IMMUNOLOGICAL AND ELECTRON MICROSCOPIC STUDY ON THE STRUCTURE OF THE ESCHERICHIA COLI RIBOSOME

Inaugural-Dissertation
zur
Erlangung des Doktorgrades
des Fachbereichs Biologie
der Freien Universität Berlin

Vorgelegt von
Marina Lotti
aus Mailand
1985

Druck: Copy-Center in Dahlem
Ladenbergstraße 2-4
1000 Berlin 33
Tel. 831 40 25

IMMUNOLOGICAL AND ELECTRON MICROSCOPIC STUDY ON THE STRUCTURE OF THE *ESCHERICHIA COLI* RIBOSOME

Inaugural-Dissertation

zur

Erlangung des Doktorgrades

des Fachbereichs Biologie

der Freien Universität Berlin

Vorgelegt von

Marina Lotti

aus Mailand

1985

1. Referent: Priv. Doz. Dr. M. Stöffler-Meilicke
2. Referent: Prof. Dr. R. Lochmann

Tag der mündlichen Prüfung: 9. 7. 1985

Für Gianfranco
und
meine Eltern

Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41548822_0039

ABSTRACT

Protein depleted core particles were prepared from both 30S and 50S subunits of *E. coli* by treatment with LiCl. The structure of the 30S core particles was characterized in detail. It was shown that these particles, which lacked one-third of their proteins, still maintained the folding and compactness of the 30S subunit, although they were less stable than the intact subunit.

Antibodies specific for ribosomal proteins which did not react with 30S and 50S subunits were, with few exceptions, also unreactive with core particles. This result indicated that the inability of many antibodies to form immunocomplexes with the ribosomal subunits may depend on the characteristics of the antibody rather than on the inaccessibility of the reactive epitopes on the surface of the intact subunits.

Six proteins were localized on the surface of the 50S subunit by specific antibodies. Proteins L5, L15 and L27 were localized on the interface side of the subunit, in a region extending from the lower part of the central protuberance to the middle of the subunit. Protein BL27 from *Bacillus subtilis* was also mapped in a comparable position as L27 on 50S subunits from a mutant of *E. coli* lacking L27 which had been reconstituted with protein BL27. Protein L9 was localized on the base of the L1 protuberance, protein L6 was mapped on the region from which the L7/L12 stalk originates and protein L4 was localized on the back side of the subunit. The specificities of all the antibodies used for the protein to be mapped were assessed by control experiments which eliminated effects due to contaminating and/or cross-reacting antibodies.

CONTENTS

		Page
1.	**INTRODUCTION**	9
2.	**MATERIAL AND METHODS**	16
2.1.	Abbreviations	16
2.2.	Buffer solutions	16
2.3.	Preparation of ribosomes and ribosomal subunits	19
2.4.	Extraction of ribosomal proteins	20
2.4.1.	Extraction with acetic acid	20
2.4.2.	Extraction with 2 M LiCl/4 M urea	20
2.5.	Preparation of 30S and 50S core particles	21
2.6.	Reconstitution of 50S subunits from *E. coli* lacking protein L27 with protein L27 from *B. subtilis*	22
2.7.	Preparation and characterization of antisera	22
2.8.	Concentration of immunoglobulins by ammonium sulfate precipitation	23
2.9.	Affinity chromatography on Protein A-Sepharose	23
2.10.	Ion-exchange chromatography on DEAE cellulose	24
2.11.	Double immunodiffusion	25
2.12.	Electrophoresis on cellulose acetate gel strips and modified immunoelectrophoresis	25
2.13.	Sucrose density gradient centrifugation	27
2.14.	Preabsorption experiments	29
2.14.1.	Preparation of protein mixtures lacking one ribosomal protein	29
2.15.	Electron microscopy	30
2.15.1.	Preparation of holey carbon films	30
2.15.2.	Carbon films	30
2.15.3.	Negative staining	31
2.15.4.	Electron microscopy	31
3.	**RESULTS: 30S CORE PARTICLES**	33
3.1.	Homogeneity of the 30S core particles as judged by sucrose gradient centrifugation	33
3.2.	Analysis of core particles and split proteins	34
3.2.1.	Protein composition of 0.5 M LiCl core particles	34
3.2.2.	Protein composition of 0.75 M LiCl core particles	36
3.2.3.	Protein composition of 1.0 M LiCl core particles	38
3.3.	Electron microscopy	40
3.3.1.	0.5 M LiCl core particles	40
3.3.2.	0.75 M LiCl core particles	41
3.3.3.	1.0 M LiCl core particles	45
3.4.	Sucrose density gradient centrifugations of 0.75 M LiCl core particles with specific antibodies	47

3.5.	Localization of proteins S11, S13 and S15 on 0.75 M LiCl core particles	51
3.6.	Reactivity of 0.75 M LiCl core particles with antibodies which do not dimerize 30S subunits	54
3.7.	Localization of protein S4 on the surface of core particles	57
4.	**DISCUSSION: 30S CORE PARTICLES**	62
4.1.	Protein composition of 30S core particles	62
4.2.	Structure of the 30S core particles	66
4.3.	Accessibility of antigenic determinants of ribosomal proteins on the surface of 30S subunits and core particles	69
4.4.	Localization of protein S4 on the surface of 30S core particles	72
4.5.	Are ribosomal proteins elongated?	74
5.	**RESULTS: TOPOGRAPHY OF THE 50S SUBUNIT**	75
5.1.	Core particles from 50S subunits	76
5.1.1.	Homogeneity of the 50S core particles as judged by sucrose gradient centrifugation	76
5.1.2.	Protein composition of 1.5 M LiCl core particles	77
5.1.3.	Electron microscopy of 50S core particles	79
5.1.4.	Sucrose density gradient centrifugations of 1.5 M LiCl core particles with antibodies specific for 50S proteins	81
5.2.	Localization of ribosomal proteins on the surface of the 50S subunit	86
5.3.	Protein L9	89
5.3.1.	Reactivity of 50S subunits with anti-L9	89
5.3.2.	Antibody specificity for protein L9	89
5.3.3.	Localization of protein L9 by immunoelectron microscopy	91
5.3.4.	Accessibility of protein L9 in the 70S ribosome	94
5.4.	Protein L6	95
5.4.1.	Reactivity of 50S subunits with anti-L6	95
5.4.2.	Antibody specificity for protein L6	95
5.4.3.	Localization of protein L6 by immunoelectron microscopy	97
5.5.	Protein L4	101
5.5.1.	Reactivity of 50S subunits with anti-L4	101
5.5.2.	Antibody specificity for protein L4	101
5.5.3.	Electron microscopy of 50S-anti-L4 complexes	103
5.6.	Protein L15	107
5.6.1.	Reactivity of 50S subunits with anti-L15	107
5.6.2.	Antibody specificity for protein L15	107
5.6.3.	Localization of protein L15 by immunoelectron microscopy	108
5.6.4.	Accessibility of protein L15 in the 70S	

	ribosome	111
5.7.	Protein L5	112
5.7.1.	Reactivity of 50S subunits with anti-L5	112
5.7.2.	Antibody specificity for protein L5	112
5.7.3.	Localization of protein L5 by immunoelectron microscopy	113
5.8.	Protein L27	117
5.8.1.	Reactivity of 50S subunits with anti-L27	117
5.8.2.	Antibody specificity for protein L27	117
5.8.3.	Localization of protein L27 by immunoelectron microscopy	118
5.8.4.	Three-dimensional localization of protein L27	121
5.8.5.	Relative locations of proteins L27 and L19 on the 50S subunit	123
5.8.6.	Reconstitution of mutant ES 5 of *E. coli* with protein BL27 from *B. subtilis*	125
6.	**DISCUSSION: TOPOGRAPHY OF THE 50S SUBUNIT**	129
6.1.	Core particles from 50S subunits	129
6.2.	Accessibility of ribosomal proteins on the surface of 50S subunits and of core particles	132
6.3.	Localization of ribosomal proteins on the surface of the 50S subunit	133
6.4.	Organization of the ribosomal proteins in the 50S subunit	135
6.5.	Comparison of the localization of proteins L4, L5, L6, L9, L15 and L27 with the results of other studies	
6.5.1.	Protein L9	137
6.5.2.	Protein L4	140
6.5.3.	Protein L6	141
6.5.4.	Protein L15	142
6.5.5.	Protein L5	143
6.5.6.	Protein L27	144
6.6.	Location of the 50S subunit proteins in the 70S ribosome	145
7.	**REFERENCES**	149
	DANKSAGUNG	151
	LEBENSLAUF	159
		160

1.INTRODUCTION

The translation of mRNA into protein occurs on ribosomes with the participation of macromolecules such as tRNA and protein synthesis factors which interact with the ribosome and with each other in a very precise manner. In order to understand this process at the molecular level, a detailed knowledge of ribosomal structure is required.

The most extensively studied ribosome is that from Escherichia coli.

Two thirds of the E.coli ribosome is RNA and one-third is protein by weight. Like other procaryotic ribosomes, it sediments with a sedimentation coefficient of 70S and has a molecular weight of 2.3×10^6 daltons. By lowering the magnesium ion concentration, the 70S ribosome dissociates into two subunits of unequal size, the small 30S (MW = 0.85×10^6 daltons) and the large 50S subunit (MW = 1.45×10^6 daltons). The small subunit consists of one 16S RNA molecule (1542 nucleotides) and 21 different proteins with molecular weights ranging from 8.000-60.000 daltons. The proteins are numbered S1-S21 on the basis of their migration in two-dimensional gel electrophoresis (Kaltschmidt and Wittmann, 1970). The large subunit is composed of two RNA molecules, the smaller 5S RNA (120 nucleotides) and the larger 23S RNA (2904 nucleotides) and 32 proteins (MW = 5.000-29.000) named L1-L34 (Kaltschmidt and Wittmann, 1970).

For historical reasons, there is a discrepancy between the nomenclature and the effective number of the large subunit proteins. It has been shown that L26 is identical to S20; upon dissociation of 70S ribosomes, 80% of this protein are associated with the small subunit, and 20% with the large subu-

nit (Wittmann et al., 1980). Furthermore, the electrophoretic spot previously referred to as protein L8, does not represent a unique protein but a complex of two dimers of L7/L12 with L10.

The complete amino acid sequences of all 53 proteins have been determined, noticeable homologies among different ribosomal proteins have not been found (Wittmann-Liebold and Dziona-ra, 1976).

The current knowledge of the secondary and tertiary structure of the ribosomal proteins has recently been reviewed (Giri et al., 1984).

The primary structures of the three RNA molecules have been determined and reliable models of their secondary structure are available (Brimacombe et al., 1983; Noller, 1984). A model for the tertiary structure of the 5S RNA has also been proposed (Pieler and Erdmann, 1982).

Each protein is present as one copy in the ribosome, with the exception of proteins L7/L12 (Pettersson and Liljas, 1979). L7 is a posttranslational modification of L12, distinct from L12 in its electrophoretic behaviour (Terhost et al., 1972). Proteins L7/L12 are present in four copies per ribosome (Wittmann et al., 1980).

The overall shape and the gross structure of the bacterial ribosome and of its subunits have been studied mainly by electron microscopy. The small subunit is an elongated particle with approximate dimensions of 230 x 110 A°; it is divided by a partition in a "head" (one-third of the particle) and a "body" (two-thirds) from which protrude two lobes of unequal size. The 30S subunit can be observed in three main projectio-

nal forms: the quasi-symmetric projection, the cloven asymmetric projection and the angled asymmetric projection. The large subunit is observed in two characteristic views, the crown view and the kidney view. In the crown view, the 50S subunit has a hemispherical shape of about 230 A° diameter with three protuberances that differ in size and shape and are referred to as the central protuberance, the wider lateral protuberance (L1 protuberance) and the rodlike appendage or L7/L12 stalk. The stalk extends 8-12 nm from the particle. The shape of both ribosomal subunits seen in different views and the current nomenclature are shown in Fig. 1. In contrast to ribosomal subunits, the 70S ribosome does not display characteristic morphological features and appears mainly as a rounded particle with approximately 230 A° diameter.

By assuming that the different views are various projections of a unique three-dimensional structure, three-dimensional models of both subunits and of the 70S ribosome have been generated by different groups. With a few exceptions, the ribosome models, after a number of years, have converged and received general acceptance. An exception is the 30S model of Lake which differs in its extreme flatness (Lake, 1976). The 50S model of Lake (1976) has received almost general acceptance and the 70S models of Stöffler (Kastner et al., 1981) and Vasiliev (Vasiliev et al., 1983) are in reasonable agreement with a model proposed by Lake (1976). Only the 50S and 70S models of Boublik deviate markedly from the general picture (Boublik et al., 1977). The models derived from electron microscopy are compatible with models obtained by small angle scattering studies (Moore, 1980; Meisenberger et al., 1984); artifacts due to specimen preparation are thus negligible. The 3D models of the 30S and 50S subunits, as well as their relative orientation in the 70S monosome as elaborated by our group are shown in Fig. 2.

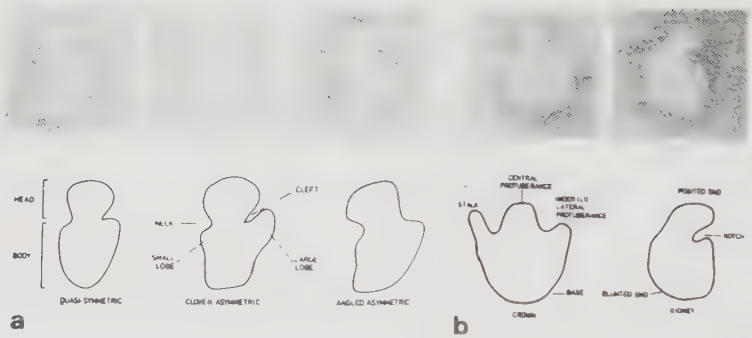


Fig.1. 30S subunits (a) and 50S subunits (b) of E. coli. Electron micrographs of both subunits seen in the characteristic projectional forms (upper row) and the correspondent diagrammatic representations (lower row), giving the nomenclature of the typical features as used in the text.

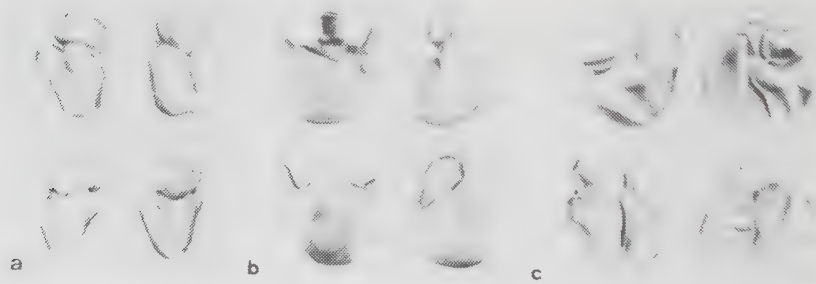


Fig.2. Three-dimensional models of the 30S subunit (a), the 50S subunit (b) and the 70S monosome (c) of E. coli

A number of techniques have been applied in the attempt to elucidate the spatial arrangement of the ribosomal proteins and RNA within the intact ribosome; immunoelectron microscopy, neutron scattering, cross-linking with bifunctional reagents, energy transfer measurement, are the most widely employed approaches to this problem (for a review see Wittmann, 1983).

In the immunoelectron microscopic studies, specific IgG antibodies are used as a label for individual ribosomal components; the divalent antibody molecule binds to its antigen within the ribosome leading to the formation of dimeric immunocomplexes which can be examined under the electron microscope. The antibody attachment site on the ribosomal surface defines the position of the antigenic determinant. The resolution of this technique is about 3.5 nm, the limiting factor being the width of the Fab arm of the antibody. Since immunoelectron microscopy was first applied to ribosomes by Wabl (1973), this method has been used by several groups to localize proteins, specific nucleotides and functional domains on the surface of ribosomal subunits (for a review see Stöffler and Stöffler-Meilicke, 1984).

Several proteins of the 30S and 50S subunit were localized by immunoelectron microscopy as early as in 1974 (Tischendorf et al., 1974 a and b). Later, it was found that some of the earlier results were incorrect, the errors arising mainly from impurities present in the antisera used for the localization. Only a small amount of the antibody raised against isolated ribosomal proteins reacts with the protein in situ. If this fraction contains antibodies in small amounts that possess a high reactivity towards ribosomes, they are likely to escape detection by the standard immunochemical methods. In order to eliminate effects of both contaminating and/or crossreacting antibodies, new control experiments were developed in our laboratory and a critical reinvestigation of the early localizations was undertaken. At the beginning of this work, altoge-

ther 15 proteins of the 30S subunit and 8 proteins of the 50S subunit had been mapped by IgGs whose specificity had been assessed with such controls.

Difficulties in the localization of the remaining proteins were met, since the available antibodies did not react with their antigen protein in situ. At the time, the most probable explanation for the lack of reactivity of several antibodies with intact subunits was that antigenic determinants of the proteins were not exposed at the surface of the intact ribosomal subunits. This hypothesis was also supported by reports of Morrison et al. (1977) and Winkelmann and Kahan (1979), suggesting that some ribosomal proteins may be inaccessible for antibody binding in the intact ribosomal subunit. For this reason, we decided to investigate whether more antigenic determinants were exposed on the surface of protein depleted core particles prepared by treatment with LiCl. In the case of positive results, a localization of the protein on the surface of the core particles should be possible. A crucial point for the feasibility of this strategy was the question of whether the core particles maintain a structural organization and a compactness comparable to those of the intact subunits. Contrasting findings have been reported in the literature about the structure of 30S core particles. On the one hand, protein deficient derivatives containing only a very few proteins had been shown to retain the main structural features of the 30S subunit (Vasiliev et al., 1977); in contrast, Boublik et al. (1982) reported that the depletion of a minimal amount of proteins caused dramatic structural alterations and unfolding of the particles. We could demonstrate that the core particles retain the structural folding characteristic of the 30S subunit, although they are less stable than the intact subunit.

In this study, 6 proteins were localized at the surface of intact 50S subunits by the use of specific antibodies. A proce-

dure based on the removal of a single ribosomal protein from a total protein mixture was developed in order to prepare the protein mixtures which are necessary for demonstrating the specificity of the antibody. For the localization of protein L27 ribosomes from a mutant of E. coli which lacks protein L27 were reconstituted with protein BL27 from Bacillus subtilis. Protein BL27 was then localized on the surface of the reconstituted 50S subunits by the corresponding antibody.

2. MATERIAL AND METHODS

2.1. Abbreviations

Tris	-	Tris(hydroxymethyl)-aminomethane
Hepes	-	hydroxyethylpiperazine-N'-2-ethanesulfonic acid
EDTA	-	ethylendiaminetetracetic acid
BICIN	-	N,N-Bis(2-hydroxyethyl)-glycine
BIS-TRIS	-	(bis(2-hydroxyethyl)iminotris (hydroxymethyl)methane)
MES	-	2(N-morpholino)ethanesulfonic acid
TP70	-	total ribosomal proteins from 70S ribosomes
TP50	-	total ribosomal proteins from 50S subunits
TP30	-	total ribosomal proteins from 30S subunits
Fab	-	fragment antigen binding of the antibody

2.2. Buffer solutions

Zamir buffer	500 mM NH_4Cl
	50 mM Tris-HCl, pH 7.8
	20 mM $MgCl_2$
	2 mM dithiotrehitol
	6 mM mercaptoethanol
PBS	0.9% NaCl
	20 mM potassium phosphate buffer pH7.8
Sucrose buffer	50 mM Tris-HCl, pH 7.8
	200 mM NH_4Cl
	$MgCl_2$ at different concentrations (Table 1)
TMA I	10 mM Tris-HCl, pH 7.8
	10 mM $MgCl_2$
	30 mM NH_4Cl
	6 mM 2-mercaptoethanol

Preparation of ribosomes and ribosomal subunits

buffer I	60 mM NH_4Cl
	20 mM Tris-HCl, pH 7.4
	10 mM $(CH_3COO)_2Mg$
	10 mM 2-mercaptoethanol
buffer II	500 mM NH_4Cl
	50 mM Tris-HCl pH 7.4
	10 mM $(CH_3COO)_2Mg$
	10 mM 2-mercaptoethanol
	1.1 M sucrose
zonal bufferI	100 mM NH_4Cl
	50 mM Tris-HCl, pH 7.4
	10 mM 2-mercaptoethanol
	0.7 mM $(CH_3COO)_2Mg$
zonal bufferII	zonal buffer I containing 40% sucrose

Preparation of core particles

buffer A	10 mM Tris-HCl, pH 7.8
	10 mM $MgCl_2$
	0.1 mM EDTA
	6 mM 2-mercaptoethanol
buffer B	10 mM Tris-HCl, pH 7.8
	10 mM $MgCl_2$
	0.1 mM EDTA
	LiCl
reconstitution buffer	360 mM KCl
	20 mM Hepes, pH 7.4
	20 mM $MgCl_2$

Electrophoresis

buffer D	0.1 M Tris-HCl, pH 8.8 0.1 M bicine 8 M urea 0.05 M 2-mercaptoethanol
buffer E	0.07 M Bis-Tris 0.07 M MES 8 M urea 0.05 M 2-mercaptoethanol
staining solution	1% Coomassie Blue R-250 in 7% acetic acid
destaining solution I	150 ml acetic acid 1000 ml methanol 850 ml H_2O
destaining solution II	70 ml acetic acid 5 ml dymethylphtalate 425 ml methanol

Double immunodiffusion

Stock solution	117.69 g barbital-natrium 77.68 g $CH_3COONa.3H_2O$ 100 mg cialit to 1000 ml with H_2O
stock buffer	200 ml stock solution 1560 ml H_2O 280 ml 0.1 N HCl
wash buffer	0.9% NaCl 10 mM Tris-HCl, pH 7.8

2.3. Preparation of ribosomes and ribosomal subunits

Ribosomes were isolated from Escherichia coli cells MRE 600 (Public Health Laboratory Service, CAMR, Porton, UK) according to Noll (1973) with some modifications (Lührmann, 1976).

For a standard preparation, 250 g of frozen cells were disrupted by grinding with 500 g of aluminium powder (Alcoa A-305) at 4°C; then, 500 ml of buffer I containing 5µg/ml DNase were slowly added. After removal of the abrasive by centrifugation at 10.000 rpm for 30 min in a Sorvall SS-34 rotor, the homogenate was further fractionated by centrifugation at 18.000 rpm for 1 h, the pellet containing cell debris and the so-called S30 supernatant containing ribosomes and soluble enzymes. In order to isolate the 70S ribosomes, aliquots of 15 ml from the S30 supernatant were centrifuged through an 8 ml sucrose cushion (20% sucrose in buffer II) in a Beckmann Ti60 rotor at 35.000 rpm for 20 hrs. The post-ribosomal supernatant (S100) was removed and the ribosomal pellet was washed three times with distilled water. Each pellet was resuspended in 2 ml buffer (in buffer I for préparation of 70S ribosomes, in zonal buffer for preparation of ribosomal subunits) and left for 6 hrs at 0°C without mechanical stirring. After resuspension, the ribosomes were centrifuged at 20.000 rpm for 15 min in a Sorvall SS-34 rotor to remove aggregated material.

30S and 50S subunits were separated by zonal centrifugation (16 hrs at 24.000 rpm) through a 6%-38% sucrose density gradient in zonal buffer in the presence of 0.7 mM $MgCl_2$ (Hindennach et al., 1971). After centrifugation, the fractions containing the 30S and 50S subunits, respectively, were pooled and the magnesium ion concentration was adjusted to 10 mM. The subunits were concentrated by centrifugation in a Beckmann Ti45 rotor at 40.000 rpm for 19 hrs. The pellets were resuspended in buffer I at a concentration ranging between

100-500 A_{260}/ml, frozen in liquid nitrogen and stored at -80°C. 250 g of cells yielded about 10.000 A_{260} 30S and 20.000 A_{260} 50S subunits.

2.4. Extraction of ribosomal proteins

2.4.1. Extraction with acetic acid (Hardy et al., 1969; Sherton and Wool, 1972).

The ribosome concentration was adjusted to 10-15 mg/ml with TMA I and the magnesium ion concentration was increased to 0.1 M by adding 1 M magnesium acetate. Then, twice the volume of cold acetic acid was slowly added to the sample which was kept in an ice bath. Thus the final concentration of acetic acid was 67% (v/v). After stirring in the cold for at least 2 hrs, the precipitated RNA was removed by centrifugation at 16.000 rpm for 30 min in a Sorvall centrifuge. The supernatant was exhaustively dialyzed against: 10% acetic acid (2 changes), 1% acetic acid containing 0.005 M EDTA (1 change), 1% acetic acid (5 changes); for the last dialysis aqua bidest was used. After dialysis, the proteins were lyophilized and stored at -20°C in the presence of silica gel.

2.4.2. Extraction with 2 M LiCl/4 M urea (Leboy et al., 1964).

The ribosome solution (10-15 mg/ml) was incubated with an equal volume of 4 M LiCl/8 M urea for 48 hrs at 4°C. The insoluble RNA was separated from the proteins by centrifugation at 16.000 rpm for 30 min in a Sorvall centrifuge. For immunodiffusion experiments, the LiCl/urea protein solution could be directly used. For other experiments, the LiCl/urea was removed by dialysis against

acetic acid as described in 2.4.1. and the proteins were lyophilized.

2.5. Preparation of 30S and 50S core particles

Core particles from ribosomal subunits were prepared according to Homann and Nierhaus (1971) with some modifications.

Ribosomal subunits were diluted to a concentration of 25 A_{260}/ml with buffer A and dialyzed for 6 hrs (3 changes) at 4°C against the same buffer. The ribosome solution was then mixed with an equal volume of buffer B containing either 1.0 M, 1.5 M or 2.0 M LiCl (for 30S subunits) or 3.0 M LiCl (for the 50S subunits) so that in the final volume the LiCl concentration was 0.5 M, 0.75 M, 1.0 M or 1.5 M. The incubation mixture was stirred overnight at 4°C. The core particles were concentrated by centrifugation through a sucrose cushion (20% in buffer B, also containing LiCl) for 7h at 45.000 rpm in a Ti60 rotor. The sediment was resuspended in reconstitution buffer, dialyzed against the same buffer for 4 hrs at 4°C (4 changes) and centrifuged at 18.000 rpm for 15 min to remove aggregated material. The LiCl extract containing the split proteins was exhaustively dialyzed against 1% acetic acid. Proteins were lyophilized and stored at -20°C.

Since core particles are very sensitive to traces of detergent and to RNase (Atsmon et al., 1969), several precautions had to be taken in order to prevent particle breakdown. All glassware was carefully rinsed to remove detergent and sterilized for 2 hrs at 160°C.

2.6. Reconstitution of 50S subunits from Escherichia coli lacking protein L27 with protein L27 from Bacillus subtilis

70S ribosomes (100 A_{260}) from mutant ES5 lacking protein L27 were dissociated into subunits by sucrose gradient centrifugation in the presence of 1 mM $MgCl_2$. Centrifugation was performed at 20.000 rpm for 16 hrs in an SW 27 rotor. The gradient fractions containing 50S subunits were pooled and, after Mg^{2+}concentration was adjusted to 10 mM, ribosomes were concentrated by centrifugation in a Beckmann Ti60 rotor for 20 hrs at 40.000 rpm. The ribosomal pellet was dissolved in 0.5 ml of 50 mM Tris-HCl pH 7.8, 20 mM $MgCl_2$, 500 mM NH_4Cl, 0.6 mM mercaptoethanol. 50 A_{260} of the 50S subunits were incubated with purified protein L27 from Bacillus subtilis in a 3-fold molar excess for 30 min at 0°C. The ribosomes were then centrifuged through a 4 ml sucrose cushion (20% sucrose in the above buffer) in a Beckmann Ti50 rotor for 7 hrs at 40.000 rpm. The pellet was resuspended in 0.5 ml of the same buffer. 37 A_{260} of reconstituted 50S were obtained.

2.7. Preparation and characterization of antisera

Immunization procedures were performed according to Stöffler and Wittmann (1971 a and b).

Antisera were characterized by double immunodiffusion (Stöffler and Wittmann, 1971 a and b), modified immunoelectrophoresis on cellulose acetate gels (Zubke et al., 1977) and immunoblotting (Towbin, 1979).

IgG immunoglobulins were purified either by gel filtration (Stöffler et al., 1973), affinity chromatography on Protein A-Sepharose (see 2.9.) or ion-exchange chromatography (see 2.10.). Some of the antibody preparations used in this study

had been purified by affinity chromatography on a column to which purified single ribosomal proteins or TP70 subfractions had been bound (Tischendorf and Stöffler, 1975).

2.8. Concentration of immunoglobulins by ammonium sulfate precipitation

10 ml of antiserum were incubated for 30 min at 55°C and then centrifuged at 15.000 rpm for 10 min in a Sorvall rotor SS-34. 6 ml of a saturated ammonium sulfate solution (760 g $(NH_4)_2SO_4$ dissolved in 1 l H_2O) were slowly added to the serum under constant stirring. Precipitation was allowed to proceed overnight at 4°C. The incubation mixture was then centrifuged for 15 min at 15.000 rpm and the sediment was resuspended in 2-3 ml of PBS and dialyzed for about 24 hrs against either PBS (for affinity chromatography on Protein A-Sepharose) or against 20 mM potassium phosphate buffer, pH 8.0 (for ion-exchange chromatography).

2.9. Affinity chromatography on Protein A-Sepharose

This procedure was used to isolate IgG antibodies from rabbit antisera and was carried out at 4°C. 15 g of dry Protein A-Sepharose CL-4B (Pharmacia Fine Chemicals, Sweden), sufficient for the isolation of IgG from a maximum of 20 ml of serum, were swollen in PBS and packed into a column (2x25 cm). The ammonium sulfate precipitate (see 2.8.) was passed through the column, collected and reapplied. The cycle was repeated three times. Then the column was exhaustively washed with PBS to remove unbound material. Elution was with 0.2 M glycine pH 2.5 , 0.15 M NaCl. When the pH in the effluent started to get acidic, the column outlet was closed. After 30 min incubation, it was opened again and 5 ml fractions were collected into tubes which contained 2 ml of 1.25 M K_2HPO_4

0.75 M NaCl in order to neutralize the glycine buffer. The column was regenerated by washing with PBS for 1 h. The fractions containing IgG were pooled, exhaustively dialyzed against 1 mM potassium phosphate buffer, pH 8.0, and lyophilized. Lyophilized IgG was stored at 4°C.

2.10. Ion-exchange chromatography on DEAE cellulose

This technique was used to isolate IgG from sheep antisera and was performed at room temperature.

15 g of diethylaminoethyl cellulose DE 52 (Whatman Chemical Separation Ltd, England) were stirred with 90 ml of 20 mM potassium phosphate buffer, pH 8.0 for 30 min. The slurry was allowed to settle and the supernatant containing any fines was decanted. The ion-exchanger was redispersed in the same buffer and the treatment was repeated until the supernatant was clear. The slurry was packed into a column of 25 x 2 cm under pressure. 20 mM potassium phosphate buffer was passed through the column until the conductivity and the pH of the effluent were the same as those of the starting buffer. This column could be used to isolate IgG from a maximum of 20 ml Of serum.

The ammonium sulfate precipitate (see 2.8.) was loaded onto the column under pressure. The fractions which were not retained and contained most of the IgG antibodies were collected, exhaustively dialyzed against 1 mM potassium phosphate buffer (pH 8.0) and lyophilized. The remaining proteins were eluted from the column by stepwise increases of the ion concentration to 60 mM, 300 mM, 1.0 M. The column was regenerated by washing with 20 mM phosphate buffer.

2.11. Double immunodiffusion

Ouchterlony double immunodiffusion tests were performed in square Petri plastic dishes (100 x 100 mm) (Stöffler and Wittmann 1971 a and b).

For preparing 10 plates, 4.5 g agarose (Serva) were heated in 200 ml of distilled water under continuous stirring until the solution was clear. Then, 100 ml Ouchterlony stock-solution were added in which 9.5 g LiCl had been dissolved. Cialit was added in traces as a preservative against bacterial growth. 30 ml gel were poured into each Petri dish and allowed to solidify at room temperature. Before use, the plates were kept for 4 hrs at 0°C. Wells were cut into the gel and the agar inside the wells was removed by a pasteur pipette connected to a water aspirator pump.

The wells were first charged with 10-80 µl antiserum and subsequentely with 10-50 µg total ribosomal proteins dissolved in 8 M urea. The wells containing ribosomal proteins were then filled with 8 M urea, those containing antiserum with PBS. Diffusion was for 48-72 hrs at 4°C in a moist chamber. The plates were washed for 2-3 days in 0.9% NaCl, 20 mM Tris-HCl, pH 7.8 to remove unprecipitated material and precipitation lines were recorded photographically, without staining.

2.12. Electrophoresis on cellulose acetate gel strips and modified immunoelectrophoresis (Stöffler, 1967; Zubke et al., 1977)

Electrophoresis was performed on cellulose acetate strips (Chemotron, Milano, Italy) in a Beckmann microzone electrophoresis cell. The cellulose acetate strips were equilibrated for a few minutes with buffer D; excess buffer

was removed with filter paper. Lyophilized ribosomal proteins were dissolved in buffer E (25-100 mg/ml) and applied to the strip with a Beckmann microzone sample applicator. Each track was loaded with 25-150 µg total ribosomal proteins. Electrophoresis was in buffer D for 90 min at 350 mV applied voltage at room temperature.

After electrophoresis, the cellulose acetate strips were washed twice for 15 min in 12.5% trichloroacetic acid to fix the proteins and then stained 1-12 hrs with 1% Coomassie Brilliant Blue R250 (Serva) in 7% acetic acid. Excess dye was removed by washing with 7% acetic acid (5 min). Destaining was performed in a solution containing 50% methanol and 7.5% acetic acid (5 min) and water was removed by washing in 100% methanol. The strips were then soaked in 85% methanol, 14% acetic acid, 1% dimethylphtalate until they were transparent (about 5 min) and finally dried on a glass plate at 80°C.

A characteristic electropherogram of total ribosomal proteins from 30S and 50S subunits and from 70S ribosomes of E. coli is shown in Fig.3.

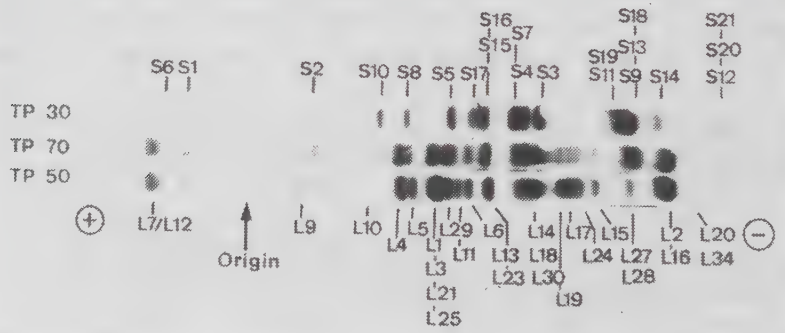


Fig.3. Cellulose acetate gel electrophoresis of TP30, TP50 and TP70 of E. coli

For modified immunoelectrophoresis, electrophoresis was as described above. Then, the strips were washed twice 1 min with PBS to remove urea and soaked overnight in antiserum specific for a single ribosomal protein. After several washes with PBS (1 hr), only insoluble protein/antibody complexes were still bound to the strip and could be stained as described above.

2.13. Sucrose density gradient centrifugation

Centrifugation on sucrose density gradients was used i) to test the homogeneity of the core particles, and ii) to separate immunocomplexes from unreacted IgG and ribosomes after reaction of the ribosomes with specific antibodies.

A 10%-30% gradient was prepared from sucrose dissolved in sucrose buffer containing the appropriate Mg^{2+} concentration (see Table 1). Ribosomes were activated for 30 min at 37°C before use. The final volume layered onto the gradient was 300 µl. Centrifugation was in a Beckmann SW40 rotor for 16-18 hrs at 18.000-23.000 rpm (see Table 1).

Antibodies specific for individual ribosomal proteins were dissolved in Zamir buffer at a concentration of 12.5 mg/ml. Aggregated material was removed by centrifugation for 2 min at 16.000 rpm in an Eppendorf centrifuge 3200. Ribosomes were added to the antibody solution at 0°C and the mixture was immediately centrifuged as in Table 1.

After centrifugation, 0.5 ml fractions were collected from the bottom of each tube. The sedimentation profiles were determined by continuously monitoring absorbance and % transmission at 254 nm (LKB 2138 Uvicord S).

Table 1. Sucrose gradient centrifugation of ribosomes and ribosome-antibody complexes

Ribosomes	A_{260}	Buffer	Mg^{2+} in sucrose	Centrifugation rpm	hrs
30S	1.0	Zamir	5 mM	23.000	16
50S	2.0	Zamir	5 mM	19.000	16
70S.I.Tight couples	3.0	Zamir	20 mM	18.000	16
70S.II.Dissociation	3.0	Noll	3 mM	18.000	16
30S-cores	1.2	Reconst.	5 mM	23.000	18
50S-cores	2.0	Reconst.	5 mM	20.000	16

n.b. Ribosome and antibody concentrations were measured in units of light absorbance at λ 260 nm and 280 nm, respectively.

2.14. Preabsorption experiments

Before addition of the ribosomal subunits, the antibody was preabsorbed with either i) purified single ribosomal protein, ii) TP 70 or iii) total protein mixtures which lack the protein in question.

Proteins were dissolved in 8 M urea and preabsorption was in 300 µl of standard sucrose buffer (5 mM Mg^{2+}) for 30 min at 37°C and for 1 h at 0°C. After incubation, any precipitated material was removed by centrifugation at 16.000 rpm for 2 min in an Eppendorf centrifuge. 250 µl of the supernatant were mixed with the ribosomal subunits and directly layered on the sucrose gradient as described.

2.14.1. Preparation of protein mixtures lacking one ribosomal protein

Protein mixtures lacking one ribosomal protein can be obtained in different ways: 1) by extraction of TP70 from mutants whose ribosomes lack a single ribosomal protein (Dabbs, 1979; Dabbs et al., 1983); 2) by mixing purified individual proteins in stoichiometric amount; 3) by immunoaffinity chromatography as described below.

1 ml Protein A-Sepharose gel (in PBS pH 7,8) was mixed with rabbit serum specific for the protein to be adsorbed. In order to remove unbound protein, the gel was washed with PBS (the washing was preferentially done in a small column). 0.25-0.5 ml TP 50 (2-4 mg/ml, extracted by the LiCl/urea method) were diluted 1:1 with PBS and mixed with the sepharose gel. After centrifugation at 3.000 rpm for 5 min, the supernatant was removed and the gel was washed with 1 ml PBS and centrifuged again. The two supernatants were pooled and directly used for

double immunodiffusion to test whether the desired protein had been quantitatively removed. Then the protein solution was dialyzed against decreasing concentrations of acetic acid as follows: 10% acetic acid (2 changes), 1% acetic acid containing 0.005 M EDTA (1 change), 1% acetic acid (4 changes), 1% acetic acid in aqua bidest (1 change).

2.15. Electron microscopy

2.15.1. Preparation of holey carbon films

A clean microscope glass slide was immersed into a cold solution of 0.2% collodium and 2.5% calcium thiocyanate in n-butylacetate. After removal of excess solution with filter paper, the film was dried by blowing on its surface. In this way a "holey" collodium film was obtained on the glass slide. The film was removed from the slide by slowly immersing the glass into a dish with distilled water. 400-mesh copper grids (Veco, Solingen-Höhscheid, Germany) were placed on the floating film and removed from the water surface by adsorption on filter paper. The grids were air dried for 12 hrs and stabilized by a thin carbon layer (Huxley and Zubay, 1960).

2.15.2. Carbon films

Thin carbon films were deposited onto the surface of freshly cleaved mica (Balzer Union, Lichtenstein) by evaporation of carbon atoms from pure graphite rods (Spektrakohle, Ringsdorf GmbH, Bonn-Bad Godesberg, Germany) performed in a high vacuum evaporator (Edwards E12E, Sussex, England). Evaporation was for 10-15 min at 10^{-15} torr vacuum. Applied voltage was 10-25 volts. The emitted carbon atoms were reflected onto the mica surface by four glass plates. By this "indirect evaporation" method very thin carbon films are obtained which are also very

homogeneous and therefore stable under the electron beam (Whiting and Ottensmeyer, 1972).

2.15.3. Negative staining

Sucrose gradient fractions containing ribosomes or ribosome-antibody complexes were prepared for electron microscopy by the double carbon layer technique as described by Tischendorf et al. (1974a).

A small square of about 2 x 2 mm was cut from a mica plate covered with a thin carbon film (2.15.2.) and immersed in 0.5 ml of the sucrose solution containing the ribosomes at a concentration of 0.2-1.0 A_{260} /ml. Depending on their concentration the ribosomes were allowed to adsorb to the surface of the floating carbon film for 10 sec - 4 min at 4°C. The carbon film was then withdrawn by lifting it with clean mica from the solution and floating it onto the surface of the staining solution. Staining was performed with 0.5% uranyl acetate or freshly dissolved 2.5% uranyl formate (pH not adjusted) for 2-3 min at 0°C .A grid coated with a holey film (2.15.1.) was placed on top of the carbon film, immediately lifted from the solution and briefly blotted on wet filter paper. Finally, the grid carrying the carbon film with the adsorbed ribosomes, was submerged under a second carbon film which had been floated on staining solution, so that the ribosomes were sandwiched between two carbon films. Grids were air dried for 12 hrs before examining them in the electron microscope.

2.15.4. Electron microscopy

Samples were examined in a Philips EM 301 or a Philips EM 400 microscope at 80 kV accelerating voltage. The grids were

inserted into the microscope with the specimen side oriented away from the electron source (Finch and Klug, 1965). Photographs were taken at an instrumental magnification of 110.000 or 100.000, respectively, on microscope sheets Planfilm 23D56 (Agfa Gevaert, Leverkusen, Germany).

3. RESULTS: 30S CORE PARTICLES

Proteins were removed from 30S subunits of *E. coli* by treatment with LiCl, following basically the procedure described by Homann and Nierhaus (1971) but with some minor modifications.

30S ribosomal subunits were incubated with 0.5 M, 0.75 M, and 1.0 M LiCl in order to obtain subparticles differing from each other in a limited number of proteins. In this way, we searched to find the optimal salt concentration which would yield homogeneous core particles suitable for immunoelectron microscopy and for studying the accessibility of ribosomal proteins *in situ*.

3.1. Homogeneity of the 30S core particles as judged by sucrose gradient centrifugation

When 1.0 A_{260} of 30S subunits, 0.5 M, 0.75 M, or 1.0 M LiCl core particles were centrifuged in a sucrose density gradient, profiles were obtained as shown in Fig.4. Each of the core particles sediments as a single peak, slightly wider than the 30S peak. Sedimentation in a narrow density region was assumed as a parameter of homogeneity. The core particles are lighter than the 30S subunits and sediment in a gradient region corresponding to lower sucrose density.

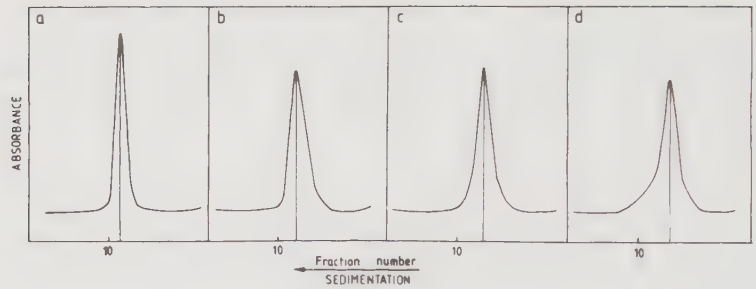


Fig.4. Sucrose density gradient centrifugation of (a) 30S subunits, (b) 0.5 M LiCl core particles, (c) 0.75 M LiCl core particles, (d) 1.0 M LiCl core particles.

3.2. Analysis of core particles and split proteins

Proteins extracted from core particles (TP cores), from control 30S subunits (TP30) as well as the split protein fraction were analyzed by: (a) electrophoresis on cellulose acetate gel strips and (b) double immunodiffusion.

3.2.1. Protein composition of 0.5 M LiCl core particles

Fig.5 shows an electropherogram of protein extracted from 0.5 M LiCl core particles (tracks 1 and 2) in comparison with total ribosomal proteins from the 30S subunit (track 3). The bands corresponding to proteins S2 and S3 are stained less than in the control, suggesting that these two proteins are present in reduced amounts in the 0.5 M LiCl core particles. The presence of protein S1 could not be unambiguously determined by electrophoresis.

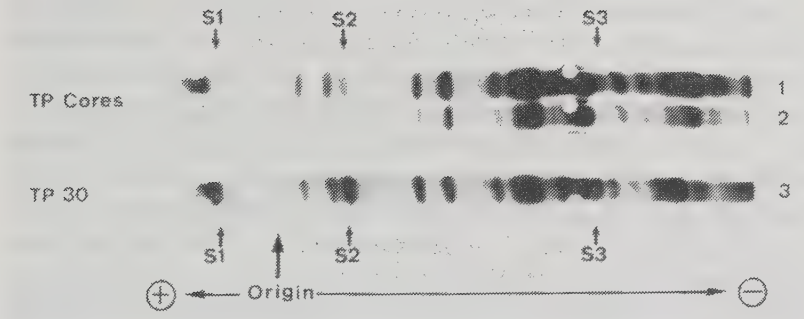


Fig.5. Cellulose acetate gel electrophoresis. Track 1: 100 µg and track 2: 50 µg of TP from 0.5 M LiCl core particles; track 3: 100 µg TP 30. Cathode and anode are indicated. Proteins which are reduced in the core particles are indicated.

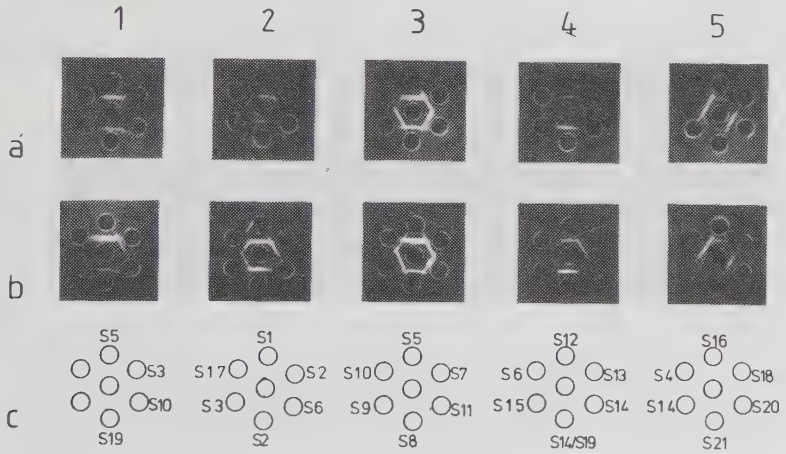


Fig.6. Double immunodiffusion. The center wells contained 15 µg TP from 0.5 M LiCl core (row a) or TP 30 (row b). The peripheral wells contained 15-80 µl of antisera specific for individual proteins of the 30S subunit, as indicated in the drawing (row c).

In double immunodiffusion experiments, TP 30 and proteins extracted from the 0.5 M LiCl core particles were reacted with antisera elicited against individual ribosomal proteins of the 30S subunit. The result of this experiment is shown in Fig.6. When TP from core particles was tested, the precipitation lines seen with antisera against proteins S1, S2 and S3 were fainter than with TP 30 (compare Fig.6 a2 with b2). In the experiment shown, no strong precipitation line is seen with anti-S6 both in TP from the cores and in TP 30; the presence of S6 in TP cores was assessed by immunodiffusion using other anti-S6 antisera. All other proteins were found to be present in the core particles in comparable amounts as in the control (compare rows a and b in Fig.6).

The results obtained from electrophoresis and immunodiffusion, taken together, led to the conclusion that in the subparticles obtained by treatment with 0.5 M LiCl proteins S1, S2 and S3 are reduced, while all other proteins are present in normal amounts (see also Table 2).

By comparing the composition of these core particles with that of another preparation obtained by 0.5 M LiCl, it was observed that core particles obtained at a given salt concentration but in different experiments can differ slightly in their protein content (Table 2).

3.2.2. Protein composition of 0.75 M LiCl core particles

The 0.75 M LiCl cores were analyzed by electrophoresis of split proteins, TP cores and control TP 30 on one and the same cellulose acetate strip (Fig.7). The bands corresponding to proteins S1, S2, S3, S5, S10 and S14 are missing or reduced in their intensity in the two tracks of TP cores (Fig. 7, tracks 3 and 4). These proteins can be seen in the tracks where split

proteins had been applied (Fig.7, tracks 1 and 2). In the split proteins, in addition, minor bands are also observed between proteins S3 and S5 and close to S14. These minor bands migrate in the positions of proteins S15/S16, S4/S7, S9/S13/S19, and S12/S20/S21, respectively (see Fig.3). Although one or more of these proteins should thus be slightly reduced in the 0.75 M LiCl core particles, the amount of reduction appears to be negligible.

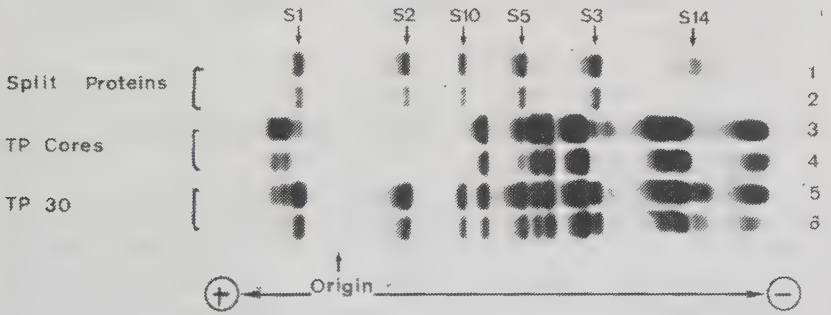


Fig.7. Cellulose acetate gel electrophoresis. Tracks 1 and 2: 50 and 25 µg of 0.75 M LiCl split proteins; tracks 3 and 4: 100 and 50 µg TP from 0.75 M LiCl core particles; track 6: 50 µg TP 30.

It was shown by double immunodiffusion experiments that in the 0.75 M LiCl core particles proteins S1, S2, S3, S10 and S14 are completely missing, while protein S5 is present in reduced amount (Table 2).

Since the core particles obtained by treatment with 0.75 M LiCl were used for several experiments it was necessary to use more than one preparation of cores. In order to obtain reproducible results, each preparation was characterized before starting experiments. As in the case of 0.5 M LiCl core par-

ticles, it was observed that various preparations differ slightly in their protein composition (Table 2). Thus another preparation of 0.75 M LiCl core particles completely lacked proteins S1, S2, S3, S5, S10, S14 and, in addition, protein S21; proteins S9 and S12 were slightly reduced. This latter preparation was used for the study on the structure of the core particles (3.3.2. and 3.5.) as well as for the experiments on protein S4 (3.7.). The core particles which lacked proteins S1, S2, S3, S10 and S14 and contained S5 in reduced amount, were used for the experiments on the accessibility of proteins to antibody binding (3.6.).

3.2.3. Protein composition of 1.0 M LiCl core particles

From the electropherogram (Fig. 8) it can be concluded that proteins S1, S2, S3, S5, S10 and S14 are completely lacking in 1.0 M LiCl core particles. Moreover, the band where proteins S12, S20 and S21 migrate is almost completely missing in TP from these cores (Fig 8).

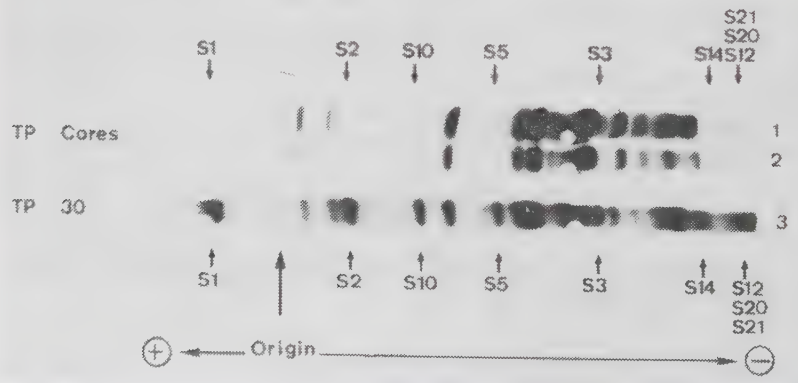


Fig.8. Cellulose acetate gel electrophoresis. Track 1: 100 µg and track 2: 50 µg TP from 1.0 M LiCl core particles; track 3; 100 µg TP 30.

Table 2 : Protein composition of core particles from 30S subunits

	0.5 M LiCl*		0.5 M LiCl		0.75 M LiCl*		0.75 M LiCl*		0.75 M LiCl		1.0 M LiCl*	
Protein	CAG	OUCH	CAG	OUCH	CAG	OUCH	CAG	OUCH	CAG	OUCH	CAG	OUCH
S1	red	red	red	red	red	-	-	-	-	-	-	-
S2	red	red	red	red	-	-	-	-	-	-	-	-
S3	red	red	red	red	red	-	-	-	red	red	-	-
S4	?	+	?	+	?	+	?	+	?	+	?	+
S5	+	+	red	red	red	red	-	-	red	red	-	-
S6	+	+	+	+	+	+	+	+	+	+	?	+
S7	?	+	?	+	?	+	?	+	?	+	?	+
S8	+	+	+	+	+	+	+	+	+	+	+	+
S9	?	+	?	+	?	+	?	±	?	+	?	-
S10	+	+	+	+	-	-	-	-	red	red	-	-
S11	?	+	?	+	?	+	?	+	?	+	?	+
S12	?	+	?	+	?	+	?	±	?	+	?	-
S13	?	+	?	+	?	+	?	+	?	+	?	+
S14	+	+	+	+	red	-	-	-	+	+	-	-
S15	?	+	?	+	?	+	?	+	?	+	?	+
S16	?	+	?	+	?	+	?	+	?	+	?	+
S17	+	+	+	+	+	+	+	+	+	+	+	+
S18	?	+	?	+	?	+	?	+	?	+	?	+
S19	?	+	?	+	?	+	?	+	?	+	?	+
S20	?	+	?	+	?	+	?	+	?	+	?	+
S21	?	+	?	+	?	*	?	-	?	+	?	-

red = protein present in the core particles in reduced amount
* = preparations of core particles which have been used for this work (see text)
? = not determined by electrophoresis

In double immunodiffusion, no precipitation lines were observed with antisera specific for proteins S1, S2, S3, S5, S9, S10, S13, S14 and S21. This result allowed the conclusion that the faint band observed in the electropherogram of the core particles in the position where proteins S12, S20 and S21 migrate corresponds to protein S20 which is contained in these core particles (Table 2). The absence of protein S9 from 1.0 M LiCl cores could not be inferred from the electropherogram.

Thus, 1.0 M LiCl core particles have lost 9 proteins, more than one-half of their protein mojety.

3.3. Electron microscopy

3.3.1. 0.5 M LiCl core particles

Electron micrographs of 0.5 M LiCl cores show particles which are uniform in their dimension and shape (Fig.9). Most of the particles possess the same morphological features characteristic for intact 30S subunits (compare Fig.9 with Fig.1).

A more detailed analysis was undertaken by evaluating 418 individual particles (Fig.9, b-f). About 70% of the core particles cannot be distinguished from intact 30S subunits and can be described as quasi-symmetric (Fig.9b), cloven asymmetric (Fig.9c), or angled asymmetric forms (Fig.9d). The relative frequency of the three projectional forms is about the same as for intact 30S subunits. In addition, projectional forms differing from those usually detected in 30S preparations, can be observed (Fig.9e-f). About 15% of the whole population appears to be altered in the region of the head; the head is either reduced in size or has indentations (Fig.9e, arrows). Some particles are thinner or somewhat smaller than

intact 30S subunits (Fig.9f, frames 1-3). Occasionally, particles are observed in which the gap between the lobe and the head is larger than in 30S subunits (Fig.9f, frames 4-5). We interpret these particles as transitional forms to the V-shaped particles which are observed in 0.75 and 1.0 M LiCl core particles (see 3.3.2. and 3.3.3.).

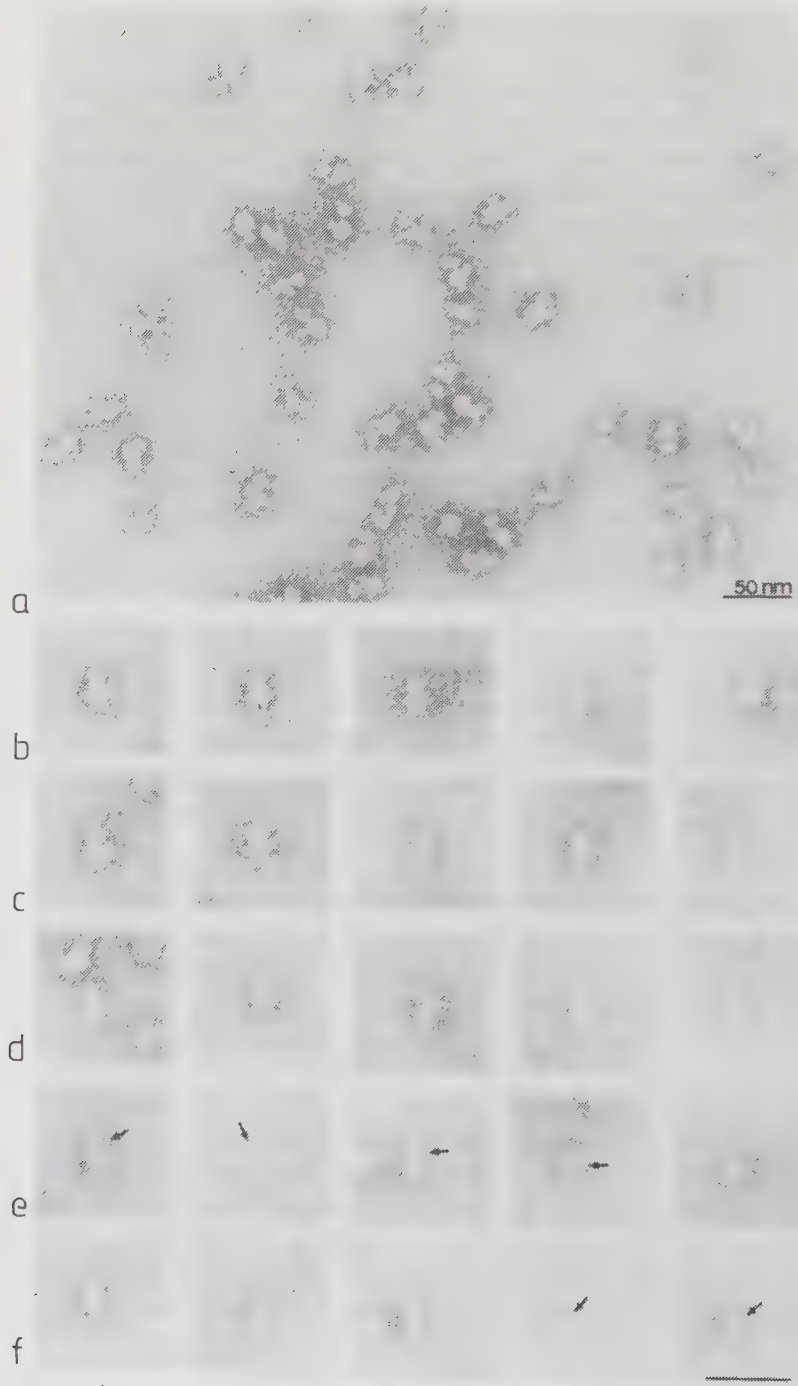
3.3.2. 0.75 M LiCl core particles

The electron microscopic investigation was performed on a core particle preparation which completely lacked proteins S1, S2, S3, S5, S10, S14 and S21 and contained proteins S9 and S12 in slightly reduced amount.

The comparison of electron micrographs of 0.75 M LiCl core particles (Fig.10) with intact 30S subunits (Fig.1) reveals strong similarities with regard to the main structural features. Most particles have the characteristic elongated shape and show the one-third/two-thirds partition. Also the side protrusions are frequently observed.

A more detailed analysis of the electron micrographs reveals an enhanced polymorphism of this preparation in comparison with 30S subunits and 0.5 M LiCl core particles (compare Fig.10 with Figs 1 and 9).

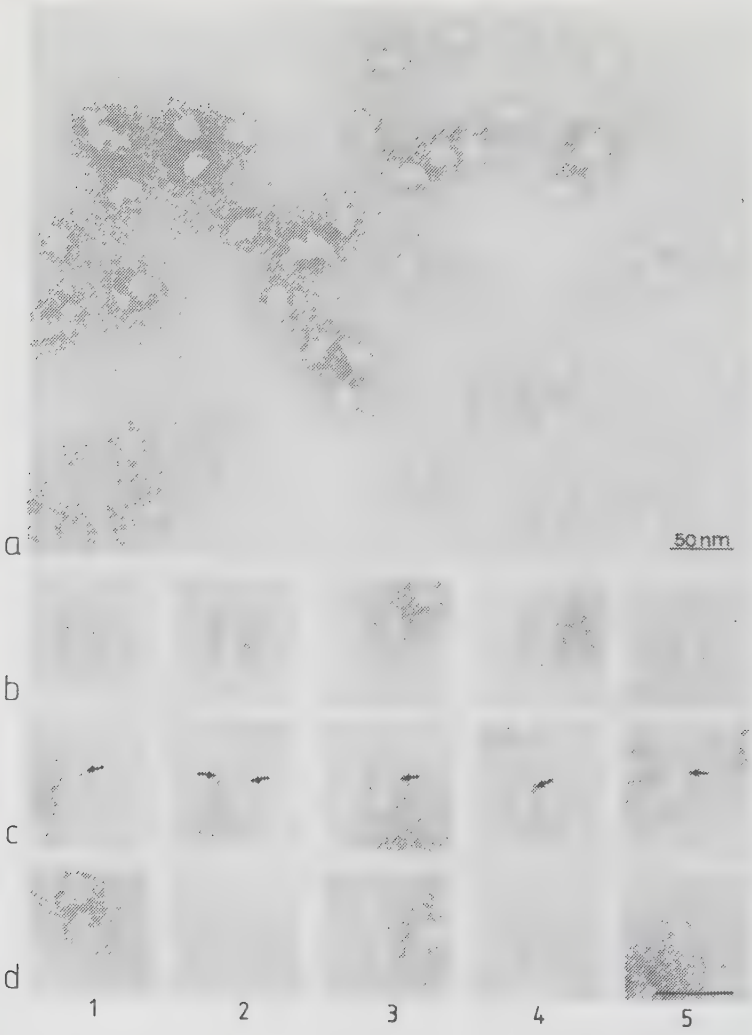
Fig.9. Electron micrographs of 0.5 M LiCl core particles. (a) General field, (b-d) selected particles resembling the intact 30S subunit, shown in the (b) quasi-symmetric, (c) cloven asymmetric, (d) angled asymmetric projectional form. (e) Particles showing alterations on their head, (f) "small" (frames 1-3) or V-shaped particles (frames 4-5).


a
50 nm
b
c
d
e
f
1
2
3
4
5

Approximately 20% of the core particles look very similar to 30S subunits (Fig.10b). Many particles show alterations in the head region, mainly reduction in size or indentations (Fig.10c), similarly as seen with some of the 0.5 M LiCl cores (comp.Fig.9e). Y- or V-shaped subparticles are seen with high frequency in the 0.75M cores. These forms are asymmetrical relative to their long axis as one of the shoulder is longer and thicker than the other. We interpret these forms as that the larger shoulder corresponds to the body of the particles ending in the head and the smaller one to the side lobe. It is likely that these structures are generated by a slight "opening" of the particle increasing the gap between the body and the large lobe. The Y- and V-shaped structures seen in the 0.75 M LiCl core preparation are similar to the projections described by Vasiliev et al. (1978) for preparations of isolated 16S RNA molecules.

In general, the 0.75 M LiCl are still quite similar to 30S subunits in their main morphological elements, but they appear to be less stable and they are seen in projections which are not observed with intact 30S subunits.

Fig.10. Electron micrographs of 0.75 M LiCl core particles. (a)General view, (b-d) selected particles. For details see text.

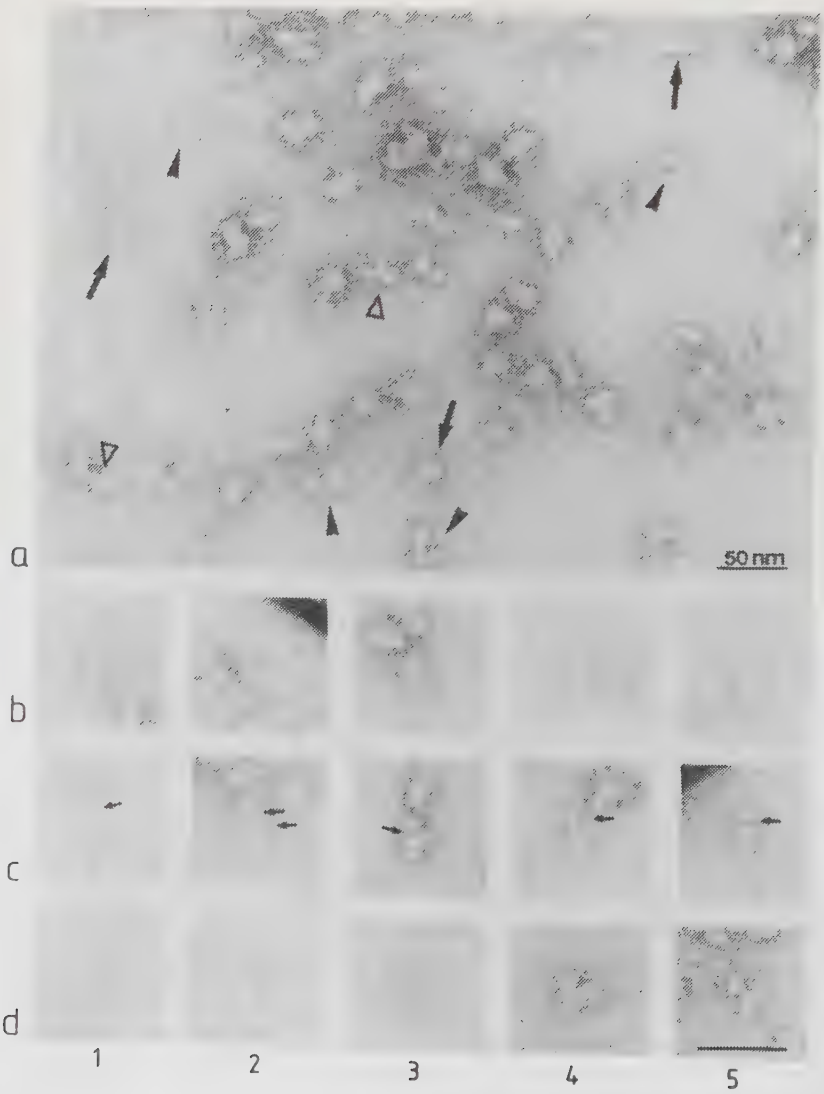

a
50 nm
b
c
d
1
2
3
4
5

3.3.3. 1.0 M LiCl core particles

As can be seen in the overall view (Fig.11a), 1.0 M LiCl core particles are rather heterogeneous in shape. Most of the particles still retain a morphological similarity with the small subunit in their main structural features, e.g. the asymmetry and the subdivision into head and body. However, the size of the particles is less uniform than in 30S ribosomes and frequently smaller particles are seen. Typical forms are indicated by different symbols in Fig.11a.

Electron microscopic images of individual particles are represented in Fig.11b-d. Particles indistinguishable from the 30S subunits have not been observed. In general, it can be said that the contours of these particles are less defined than in intact 30S subunits, even in those particles retaining a 30S like structure (Fig.11b). Protein depletion seems to effect mainly the structural organization of the head although in most cases it is still clearly recognizable (Fig.11c, arrows). Y- and V-shaped forms, similar to those observed with 0.75 M LiCl cores are frequently detected (compare Fig.11d with Fig.10d).

Fig.11. Electron micrographs of 1.0 M LiCl core particles. (a) General field in which some typical forms are indicated by arrows: particles smaller than the 30S subunit (➡), particles with alterations in the head (▷), V-shaped particles (➡). (b-d) Selected micrographs of characteristic forms.


a
50 nm
b
c
d
1
2
3
4
5

3.4. Sucrose density gradient centrifugations of 0.75 M LiCl core particles with specific antibodies

Since the core particles obtained by 0.75 M LiCl treatment completely lacked 1/3 of their proteins (see 3.2.2.) and yet retained a well defined morphology (see 3.3.2.), they were used for all further experiments.

First, sucrose gradient centrifugation was used to test whether epitopes of proteins S3, S5, S10 and S14 which are missing in the 0.75 M LiCl cores, are really absent from the surface of the subparticles. These experiments were carried out with antibodies whose specificity and extent of reactivity with intact 30S subunits had previously been assessed. The 0.75 M LiCl core particles did not dimerize with any of the four IgG preparations tested, while the same antibodies strongly reacted with 30S subunits in control experiments (Table 3). The presence of epitopes of proteins S1, S2 and S21, which are also missing in the 0.75 M LiCl cores, could not be tested, because at the time antibodies specific for these three proteins were not available.

These experiments confirmed the results obtained by immunodiffusion and electrophoresis (3.2.2.), namely that proteins S3, S5, S10 and S14 are absent from the 0.75 M LiCl cores; they furthermore excluded that any of the four proteins are present on the subparticles in small amounts only.

Next, the subparticles were reacted with antibodies directed against those proteins which are contained in the 0.75 M LiCl core particles, the so-called "core" proteins. These experiments were carried out with antibody preparations which were reactive with 30S subunits and had been used to localize the corresponding proteins. For almost all the antibody tested the

Table 3. Reactivity of 0.75 M LiCl core particles and 30S subunits with antibodies specific for "split" proteins

Antibody to protein	Antibody preparation	A_{280}	Dimer formation 30S	cores
S3	H123 II IgG	1.0	35%	-
S5	H15 ACG SPS5 Pool	0.3	35%	-
S10	H145 Eluat SPS10 DEAE cut 1/2	1.0	27%	-
S14	H134 IgG	2.0	34%	-

Table 4. Reactivity of 0.75 M LiCl core particles and 30S subunits with antibodies specific for "core" proteins

Antibody to protein	Antibody preparation	A_{280}	Dimer formation 30S	cores
S4	H24 DEAE Trisacryl cut 1	0.3	20%	22%
S7	H156 IgG	2.0	30%	30%
S11	R222 ACG SPS11	1.0	25%	30%
S13	R143 I IgG	0.4	22%	20%
S15	ACG RPool ex SPS17, SPS18	0.3	23%	25%
S16	ACG RPool	0.5	50%	50%
S18	H66 ACG SPS18 Pool	0.3	45%	47%

amount of dimerization with the core particles was comparable to that with 30S subunits (Table 4).

However, the reaction of antibody specific for protein S11 resulted in the formation of a peak, sedimenting more slowly than the peak of the core particles, as shown in Fig. 12. A possible explanation for this effect is that a subfraction of the population of the cores undergoes breakdown.

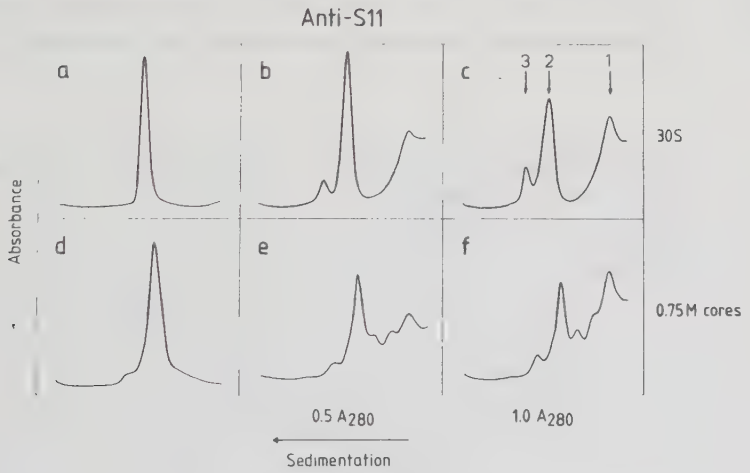


Fig.12 Sucrose density gradient centrifugation of 30S subunits and 0.75 M LiCl core particles with increasing amounts of anti-S11. Peak1: IgG; peak 2: monomers; peak 3: immunocomplexes.

When 0.75 M LiCl core particles were reacted with anti-S6 antibody, no dimerization was observed. This was surprising, since protein S6 is contained in the cores (see Table 2). Therefore, other IgG preparations specific for protein S6 were tested for their reactivity with the core particles (Table 5). It was observed that two of the antibodies were

reactive with 30S subunits but not with core particles. In contrast, two other anti-S6 preparations formed comparable amounts of dimers with 30S and core particles (Table 5). An explanation for the results obtained with anti-S6 from H 58 and R 1124 could be that these antibodies pulled S6 off the ribosome and therefore no dimerization was seen.

These experiments showed that the removal of proteins from the 30S subunit does not increase the extent of dimerization of the core particles, when reacted with antibodies to individual proteins. Therefore, we drew the conclusion that no additional epitopes of the proteins we studied are exposed on the surface of the core particles.

Table 5. Reactivity of 30S subunits and 0.75 M LiCl core particles with anti-S6 antibodies.

antibody preparation	A_{280}	Dimer formation	
		30S	cores
H58 Eluat II SPS6/SPS2	0.1	44%	-
R1124 IgGa I	0.25	25%	-
R1125 IgGa I	1.0	37%	33%
H160 DEAE cut 1/2	0.2	15%	15%

3.5. Localization of proteins S11, S13 and S15 on 0.75 M LiCl core particles

In order to rule out a possible loss of internal organization, which would be difficult to appreciate from the shape and contours of the particles, some of the core proteins were localized on the surface of the core particles by immunoelectron microscopy and the antibody binding sites observed were compared with those reported for 30S subunits. If no important structural changes or rearrangements had occurred within the protein depleted particles, antibody binding should be observed in comparable areas on the surface of both 30S subunits and core particles.

Antibodies against proteins S4, S11, S13 and S15 were used as markers of the main structural features of the 30S subunit: the head, the body and the two side lobes. The localization reported for these four proteins on the surface of the 30S ribosome is summarized in Fig.13.

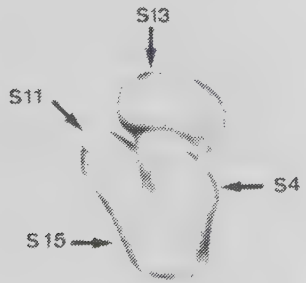


Fig.13. Location of proteins S4, S11, S13 and S15 on the three-dimensional model of the 30S subunit (Tischendorf et al., 1975; Stöffler-Meilicke et al., 1984; Stöffler and Stöffler-Meilicke, 1984).

When complexes of core particles with anti-S11, anti-S13 and anti-S15 were observed in the electron microscope, dimers in which both subparticles were observed in interpretable forms were very rare. Selected electron micrographs of pairs of core particles connected by anti-S11, anti-S13 and anti-S15 are shown in Fig.14; the antibody binding sites on the subparticles can be compared with those on intact 30S subunits, which are shown for comparison. The localization of protein S4 will be discussed discussed below (see 3.7.).

As on 30S subunits, antibody specific for protein S11 binds to the large lobe of the core particles (Fig.14). The IgG molecule is always visible in its full length, indicating that this protein is localized at the tip of the lobe. Most immunocomplexes formed by anti-S11 both with 30S subunits and with core particles, have two antibody molecules bound simultaneously. Thus, at least two epitopes of this protein are accessible to antibody binding in both 30S subunits and 0.75 M LiCl cores. However, the two antibody binding sites are very close to each other and do not suggest that protein S11 has an elongated shape in situ.

IgG specific for protein S13 attaches to the top of the head both in core particles and in intact 30S subunits (Fig.14). Pairs of subunits connected by two IgG molecules have not been observed. In the subparticles, no additional binding sites have been observed.

Protein S15 has been localized on the body of the 30S subunit, below the large lobe. Electron micrographs of core particles reacted with anti-S15 show the same antibody binding sites (Fig.14). Exposure of additional epitopes has not been observed in the core particles.

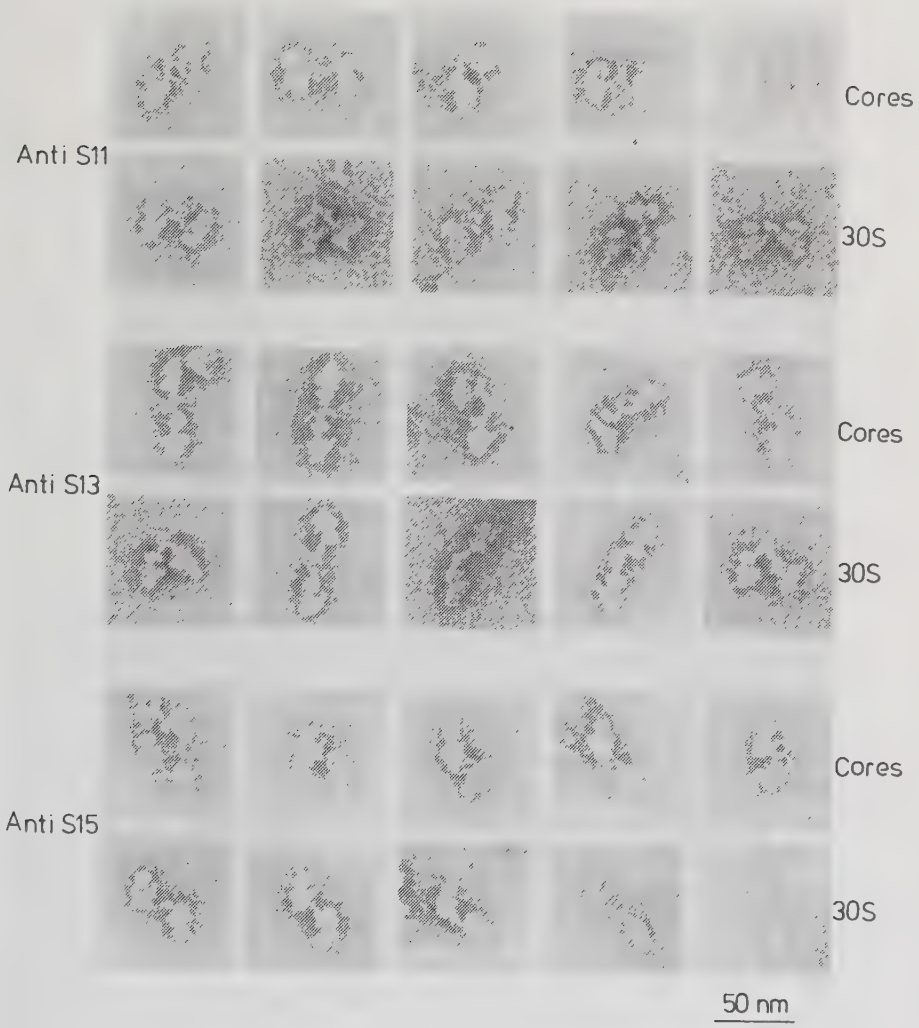


Fig.14. Electron microscopic localization of proteins S11, S13 and S15 on the surface of 0.75 M LiCl core particles and 30S subunits.

3.6. Reactivity of 0.75 M LiCl core particles with antibodies which do not dimerize 30S subunits

In the preceding sections it was shown that core particles depleted of a substantial part of their proteins by treatment with LiCl still retain a well defined structure and are therefore suitable for studies on the structure of the 30S subunit. Our main aim in undertaking this study was to investigate the reactivity of core particles with antibodies which do not form complexes with complete 30S subunits. Many antibodies specific for individual ribosomal proteins, among them all the available preparations raised against proteins S1, S8, S9, S12 and S20, did not react with 30S subunits in sucrose gradients. For this reason it has not yet been possible to localize these four proteins on the 30S subunit.

Thus, antibodies specific for proteins S8, S9, S12 and S20 were tested for their reactivity with 0.75 M LiCl core particles in order to investigate if these antibodies react with antigenic determinants which are not exposed in the 30S subunit (see Table 6). Antibodies to protein S1 were not tested since S1 is removed by treatment with LiCl. Proteins S4, S16 and S17 have already been localized on the surface of 30S subunits. However, several antibodies specific for these three proteins did not react with 30S subunits. These IgGs were tested for their reactivity with core particles in order to investigate whether additional epitopes of S4, S16 and S17 are exposed at the surface of the core particles. For this study a preparation of core particles was used which lacked proteins S1, S2, S3, S5, S10 and S14 (see 3.2.2.). With one exception, for all the IgG preparations tested, no dimer formation was observed with the core particles. A reduction of the monomer peak, which might also be an indication for reactivity of the antibody, was not observed either. Thus, the depletion of 1/3 of the proteins does not exert any noticeable effect on the reactivity of the core proteins with antibodies directed against them.

Table 6. Reactivity of 0.75 M LiCl core particles with IgGs which do not dimerize 30S subunits

Antibody to protein	Antibody preparation	A_{280}	Dimer formation 30S	cores
S4(F)	H197 IgG	3.0	-	15%
S4(F)	H194 DEAE cut 1/2	3.0	-	-
S4(F)	H195 DEAE cut 1/2	3.0	-	-
S4(F)	H196 DEAE cut 1/2	3.0	-	-
S8	H149 DEAE cut 1/2	3.0	-	-
S8	R1013 IgGA	3.0	-	-
S8	ACG TP50 ex SPS8	1.5	-	-
S9	R1109 IgGA	1.5	-	-
S9	H121 ACG SPS9	0.5	-	-
S12	H18 Eluat cut B ex IgG+ACG	3.0	-	-
S13	H109 IgG	1.5	5%	5%
S16	H150 DEAE cut 1/2	1.5	-	-
S17	H144 ACG SPS17	0.4	-	-
S20	H63 Eluat TP50,TP30 ACG SPS20	2.0	-	-
S20	R191 Eluat TP70 V9-4	2.0	-	-
S20	RPool IgGA	3.0	-	-

The only antibody which was found to be reactive with the core particles was the IgG preparation raised in sheep 197 against a fragment of protein S4 (Table 6). This result indicates that this antibody reacts with an antigenic determinant which is not exposed in the 30S subunit. Now, it was of interest to study which proteins are responsible for the masking of the reactive epitope in the complete 30S subunit. Other authors have suggested that proteins S5 and S12 cover the reactive epitope of protein S4 within the complete 30S subunit (Winkelmann and Kahan, 1979). Therefore, we reacted the anti-S4 antibody also with 0.5 M core particles which contain the only proteins S1, S2 and S3 in reduced amounts. With 0.5 M core particles were formed immunocomplexes in comparable amounts as with 0.75 M LiCl core particles (Fig.15). This epitope was localized at the surface of the 0.5 M core particles at the same position as the epitope of protein S4 on intact 30S subunits (see 3.7.).

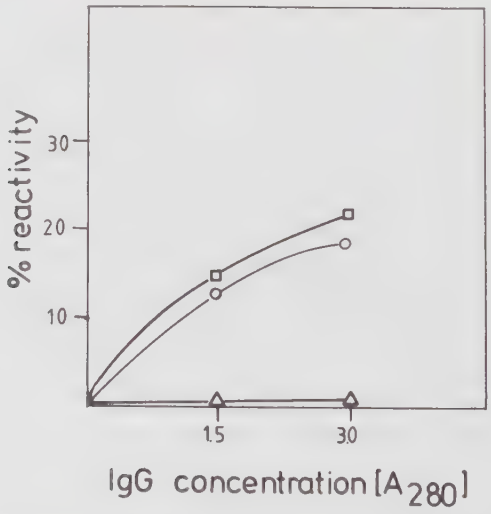


Fig.15. Reactivity of an antibody specific for a fragment of protein S4 with : 30S subunits (Δ—Δ), 0.5 M LiCl core particles (o—o) and 0.75 M core particles (□—□).

3.7. Localization of protein S4 on the surface of core particles

The location of protein S4 on core particles has been investigated in detail, because of the serious disagreement in the literature about the question of whether epitopes of ribosomal protein S4 are accessible for antibody binding on the intact small ribosomal subunit (for discussion see Stöffler-Meilicke et al., 1984). An antibody preparation specific for protein S4 had been obtained in our laboratory and was shown to react with 30S subunits. This antibody was used for the localization of protein S4 on the surface of 30S subunits (Stöffler-Meilicke et al., 1984). In order to investigate whether additional epitopes of protein S4 are exposed after the depletion of proteins by LiCl, the same antibody was tested for its reactivity with core particles.

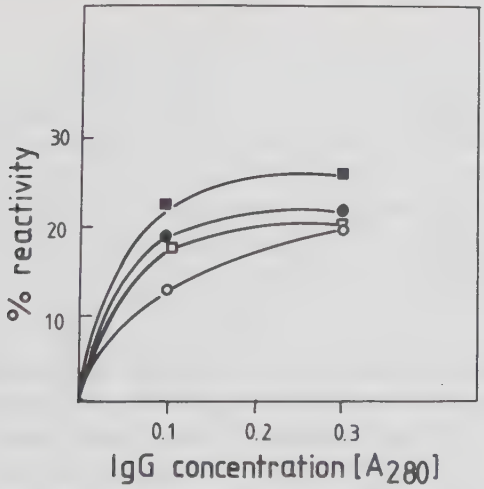


Fig.16. Antibody-ribosome complex formation with anti-S4. Increasing amount of IgG were reacted with: 30S subunits (o—o), 0.5 M LiCl core particles (□—□), 0.75 M LiCl core particles (●—●) and 1.0 M LiCl core particles (■—■). Reactivity was calculated as percentage of dimerized particles plotted against antibody concentration.

The reactivity of increasing amounts of anti-S4 IgG with 30S, 0.5 M, 0.75 M and 1.0 M LiCl core particles is shown in Fig.16. At high antibody concentration, approximately 20% of the 30S subunits were reactive with this antibody. A slight increase in the extent of dimerization can be observed with the core particles (Fig.16). Since depletion of proteins by treatment with LiCl does not considerably increase the reactivity of the particles with anti-S4, it can be concluded that the number of epitopes of protein S4 exposed on the surface of the core particles cannot be significantly larger than on intact 30S subunits.

The antibody binding site of anti-S4 on 30S subunits and 0.5 M and 0.75 M LiCl core particles was investigated by electron microscopy. Overall views and characteristic immunocomplexes are shown in Fig.17.

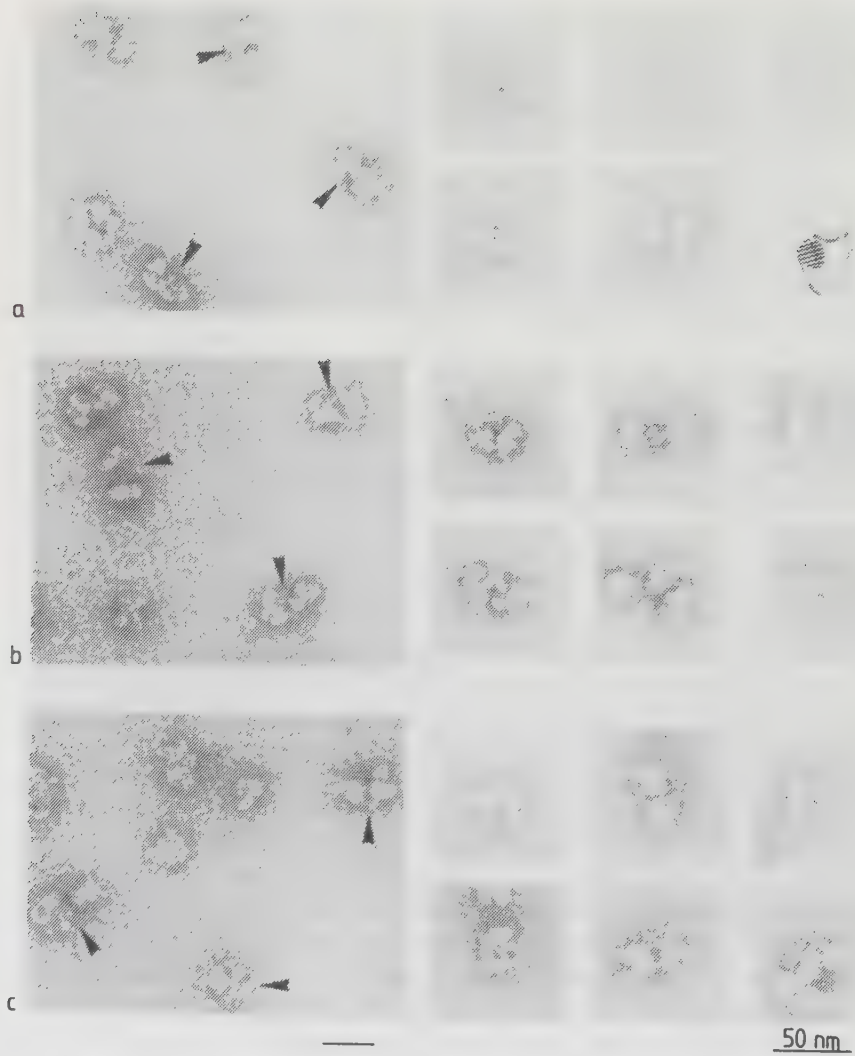
With 30S particles, 80% of the subunits are present as 30S-IgG-30S dimers (Fig.17a). In the immunocomplexes, the subunits are mainly very close to each other and therefore the Fab arms of the connecting IgG molecules are not fully visible. Pairs of 30S subunits connected by two antibody molecules are frequently observed. Antibody binding is on the small lobe of the subunit.

Fig.17b shows a general field and a gallery of individual immunocomplexes of 0.5 M LiCl core particles reacted with anti-S4. The antibody attachment site can be easily recognized because of the morphological similarity of the 0.5 M LiCl subparticles with 30S subunits (3.3.1.). The IgG molecules bind to the small lobe, close to the one-third/ two-thirds partition. Pairs of particles simultaneously connected by two antibody molecules are frequently observed; however, they are not more frequently seen than with 30S subunits. Antibody binding differing from that found with 30S subunits was not ob-

served.

If anti-S4 is reacted with 0.75 M LiCl core particles, dimers in which the shape of the subparticles can be recognized are seen very rarely. From all interpretable forms, we concluded that the antibody binding site is also on the small lobe (Fig.17c). Pairs of subparticles joined by two antibody molecules are frequently observed. Binding sites other than those seen with 30S subunits and 0.5 M LiCl core particles are not detected (Fig.17c).

Fig.17. Electron micrographs of anti-S4 reacted with (a) 30S subunits, (b) 0.5 M LiCl core particles, (c) 0.75 M LiCl core particles. In the general views typical immunocomplexes are marked by arrowheads. In row (d) are represented some individual complexes of 0.5 M LiCl core particles reacted with an antibody specific for a fragment of protein S4. The location of protein S4 on the three-dimensional model of the 30S subunit is given in the last frame of Fig. 17a.


a
b
c
50 nm
d

In Fig.17d are shown complexes of 0.5 M LiCl core particles reacted with an IgG specific for a fragment of protein S4. This antibody was not reactive with intact 30S subunits but formed immunocomplexes with 0.5 M and 0.75 M LiCl core particles (3.6.). Also for this antibody the binding site was on the small lobe.

From these results it was shown that protein S4 is exposed for antibody binding both in the 30S subunits and in 0.5 M and 0.75 M LiCl core particles. However, some antigenic determinants of S4 are not accessible in intact 30S subunits but are accessible in 0.5 M and 0.75 M LiCl cores.

There is still some debate about the question of whether protein S4 is elongated in the ribosome. Early immunoelectron microscopic investigations suggested a highly elongated shape for protein S4 (Lake et al., 1974; Tischendorf and Stöffler, 1975). However, we observed the same antibody binding site on the surface of 30S subunits and protein depleted core particles. In addition, antibody specific for an epitope of protein S4 which is not accessible in the 30S subunit (3.6.) binds to the surface of 0.5 M LiCl core particles in the region of the small lobe . The above described results indicated that protein S4 is not elongated.

4. DISCUSSION: 30S CORE PARTICLES

4.1. Protein composition of 30S core particles

From the analysis of the protein contents of core particles obtained from 30S subunits, we observed that the proteins are removed by LiCl treatment according to their order of assembly: the proteins which are easily depleted by salt are late assembly proteins and interact in their assembly in the 30S subunit (Fig.18b).

Most of the proteins which are depleted by LiCl are neighbours in the three-dimensional structure of the small subunit. Fig.18a shows the location of the proteins removed by treatment with 0.75 M LiCl in relation to the other ribosomal proteins. Proteins S2, S3, S10 and S14 have been localized by immunoelectron microscopy on the head of the subunit, on the side of the small lobe. Protein S5 has been mapped on the small lobe, close to the neck region; S21 has been located in the cleft (Stöffler and Stöffler-Meilicke, 1984; Schwedler-Breitenreuter et al., 1985). Proteins S3, S5, S10 and S14 have also been localized in one domain by neutron scattering (Ramakrishnan et al., 1984). Protein S1 has not yet been mapped by immunoelectron microscopy but it has been located by neutron scattering studies close to proteins S3, S5 and S10 (Ramakrishan et al., 1981). Thus, the proteins which are missing in the 0.75 M LiCl core particles and which constitute about 46% of the total proteins of the small subunit, are all located in a single structural domain with the only exception of protein S21 which is located at the large lobe (Fig.18a). Proteins S2, S3, S10 and S14 also constitute a functional entity in the ribosome, being involved in the binding of tRNA to the ribosomal A-site (Randall-Hazelbauer and Kurland, 1972; Lelong et al., 1979).

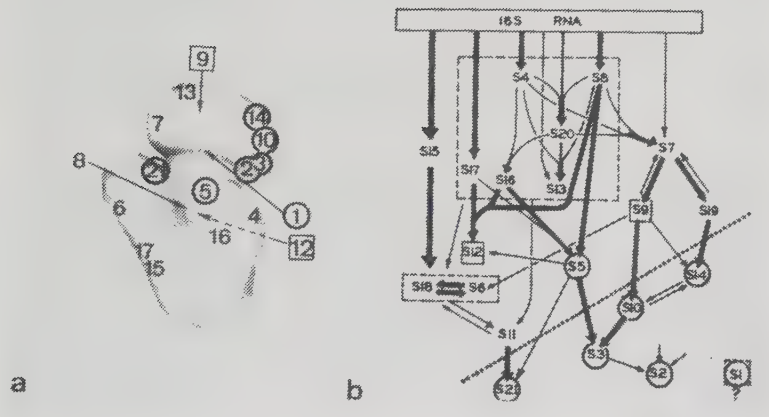


Fig.18. 3-D model (a) and assembly map (b) of the 30S subunit showing the position of the proteins removed by 0.75 M LiCl (circles). By 1.0 M LiCl S9 and S12 are further depleted (squares). The assembly map is from Mizushima and Nomura (1970). The location of the 30S proteins is according to Stöffler and Stöffler-Meilicke (1984). S12 has been localized by Lake (1980). The position of protein S9 has been determined by neutron scattering (Ramakrishnan et al., 1981).

If the salt concentration is increased to 1.0 M, proteins S9 and S12 are also removed. These two proteins have assembly interactions with proteins S5, S10 and S14 and are close neighbours of the other split proteins (Fig.18).

30S ribosomal subunits partially depleted of their proteins have been widely used in studies on the assembly of the 30S

subunit (Mizushima and Nomura, 1970; Homann and Nierhaus, 1971) and on the role of the 16S RNA and of the ribosomal proteins in determining the shape of the subunit (Vasiliev and Koteliansky, 1977; Vasiliev et al., 1977; Boublik et al., 1982). Therefore, core particles obtained by different methods have been characterized and described in the literature.

Significant differences are observed if one compares the protein content of 1.0 M LiCl cores obtained in different laboratories (Table 7). Whereas proteins S1, S2, S3, S9, S10, S12 and S14 are missing or present in reduced amounts in each preparation, differences are observed for other proteins (Table 7). Thus, the protein content of the core particles prepared by Homann and Nierhaus, differ from the others in that they contain protein S5 but lack S11 and S18 (Table 7). On the other hand, the core particles described by Boublik et al., have reduced amounts of proteins S4 and S8 (Table 7).

Most authors emphasize that even the protein content of core particles, prepared by the same procedure can slightly vary in different preparations. This effect has also been observed with our core preparations (Table 2). Therefore, the protein content of each LiCl core preparation has been determined and each set of experiments has been performed with one and the same preparation, in order to obtain unambiguous results.

Table 7. Variation of the protein content of 1.0 M LiCl core particles prepared in different laboratories

Protein	(a)	(b)	(c)
S1	-	red	red
S2	-	-	red
S3	-	-	-
S4	+	red	+
S5	-	red	+
S6	+	red	+
S7	+	+	+
S8	+	red	+
S9	-	-	-
S10	-	-	-
S11	+	-	-
S12	-	-	-
S13	+	+	red
S14	-	-	-
S15	+	+	+
S16	+	+	+
S17	+	-	+
S18	+	n.d.	-
S19	+	n.d.	+
S20	+	n.d	red
S21	-	n.d.	-

red = protein detected in reduced amount
n.d.= data not determined

(a) this work; (b) Boublik et al., 1982; (c) Homann and Nierhaus, 1971.

4.2. Structure of the 30S core particles

Our investigation of the core particles by electron microscopy showed that all three classes of subparticles maintained the main morphological features typical for the small ribosomal subunit, such as the elongated shape, the partition into head and body, and the presence of the two side lobes. Nevertheless, the depletion of proteins induced a loss of stability of the particles, generating various forms which are not seen in 30S preparations. Alterations in the head region were frequently observed. This is not surprising if one considers that most of the split proteins are removed from the head region of the 30S subunit (Fig.18a). With increasing salt concentration, Y-shaped forms were observed more frequently. These forms could be explained by unfolding of the particles, resulting in an enlargement of the cleft which divides the head from the large lobe. These Y-shaped particles were very similar to isolated 16S RNA molecules characterized by Vasiliev et al. (1978).

On the basis of immunochemical and electron microscopic analysis, the subparticles obtained by treatment with 0.75 M LiCl were judged to be the most suitable for structural studies since they are depleted of seven proteins but still retain a well defined structural organization similar to that of intact 30S subunits. The location of proteins S4, S11, S13 and S15 on the surface of the subparticles at the same site found with 30S subunits excluded changes in the internal organization of the particles (see 3.5.). This finding is of general interest for the study on the location of ribosomal proteins by antibody label, particularly in those cases in which the IgG react only with a small fraction of the subunit population, possibly consisting of functionally inactive particles. It has been reported that 30S subunits differing in their activity in protein biosynthesis have different structures, based on electron microscopic observations (Myazawa et al.,

1979). This finding could suggest that the arrangement of the ribosomal proteins is different in active and inactive subunits. Our results, however, demonstrate that the relative arrangement of the ribosomal proteins is the same in both active 30S subunits and core particles and therefore the degree of activity of the subunits is not determinant for our topographical studies.

Much effort has been focussed on the study of the structure of core particles from 30S subunits in the attempt to elucidate the role of the RNA and the ribosomal proteins in determining the shape of the subunit. In fact, if the RNA determines the ribosomal shape, the depletion of proteins should not induce relevant changes in the structure of the particles; if, on the contrary, the proteins have an important structural role, their removal by salt will induce unfolding of the particles.

Contradictory results have been reported about the shape and structural organization of protein depleted particles. Vasiliev's group performed a series of experiments in which either 30S subunits were depleted of a large number of proteins (Vasiliev and Koteliansky, 1977; Vasiliev et al., 1977a) or nucleoprotein particles were reconstituted from 16S RNA and ribosomal proteins (Vasiliev et al., 1977b; Vasiliev, personal communication). The shape of these particles was studied in the electron microscope using freeze-drying and heavy metal shadowing as the preparation technique. The particles were suspended in a buffer containing 1.0 M ethanol. By treatment with 2.15 M LiCl they obtained ribonucleoprotein derivatives with a sedimentation coefficient of 25S, lacking 12 proteins (Vasiliev and Koteliansky, 1977). These particles showed no significant differences in either their size or their morphology, if compared with 30S subunits. Moreover, the RNA in the subparticles had the same compactness and packing as within the 30S subunit (Serdyuk, 1983). Even core particles sedimenting with 22S and containing just proteins S4, S7, S8, S15 and

S16 still maintained the compactness and the main morphological features of the ribosomal particle, if they were prepared in the presence of 1.0 M ethanol (Vasiliev et. al, 1977a). Under the same conditions, even complexes of 16S RNA and S4 showed a morphology similar to that of the 30S subunit, although less rigid, sometimes showing V-shaped and Y-shaped particles (Vasiliev et al., 1977b). Finally, it was shown that under certain ionic conditions the 16S RNA is capable of self-packing into a specific compact structure bearing morphological features of the 30S ribosomal subunit (Vasiliev et al., 1978). These observations, supported by physical studies (Serdyuk, 1979 and 1983) led to the concept that the 16S RNA is the main element in determining the ribosomal structure. Thus the RNA would provide a three-dimensional framework for the arrangement of the ribosomal proteins.

Contrasting observations have been reported by Boublik et al. (1982) in a study on the structure of core particles obtained by treatment of 30S subunits with 0.3 to 3.0M LiCl. In this study the depletion of proteins S2, S3 and S10 achieved by 0.3 M LiCl led to a loss of the characteristic features of the 30S subunit. This effect was enhanced if more proteins were depleted. In general the core particles are described as unfolded and polydisperse in size and shape.

If the structure of the 0.3M core particles described by Boublik is compared with those prepared for this work at similar salt concentrations, it is evident that even our 0.75M LiCl core particles have a better structural compactness although they are devoid of a larger number of proteins (Table 2). With regard to their structure, the 0.3 M LiCl core particles of Boublik are rather similar to our 1.0 M subparticles which completely lack 9 of their proteins.

The different findings which have been reported about the fol-

ding of 30S core particles could be due, at least in part, to the different experimental conditions used. Thus, for example, the cores of Vasiliev and ours were dissolved in a reconstitution buffer, whereas those of Boublik were in a standard ribosome buffer at low ionic strength.

From our own observations, we draw the conclusion that the protein depleted subparticles still retain the structural folding characteristic for the 30S subunit, but they are less stable than the intact subunit. In conclusion, it seems likely that the 16S RNA play an essential role in determining the shape of the 30S subunit. On the other hand, the instability of the core particles supports the concept that the ribosomal proteins are important for stabilizing the three-dimensional structure.

4.3. Accessibility of antigenic determinants of ribosomal proteins on the surface of 30S subunits and core particles

The accessibility of the 30S subunit proteins to antibody has been investigated in detail by immunological methods (Stöffler et al., 1973; Stöffler, 1974; Zeichhardt, 1976). Quantitative differences between the degree of accessibility were observed for different proteins. These studies supported the concept that most proteins have at least one epitope exposed at the surface of the ribosomal subunit. This conclusion was supported by other data, such as the sensitivity of ribosomal proteins to enzymatic attack (Craven and Gupta, 1970; Chang and Flask, 1971; Spitnik-Elson and Breiman, 1971; Crichton and Wittmann, 1971) or the modification of ribosomal proteins by chemical agents (Craven and Gupta, 1970).

Nevertheless, there is still disagreement in the literature

about the question of whether protein S4 is exposed at the surface of intact 30S subunits (see also 4.4.). Moreover, up to now we have failed to map proteins S1, S8, S9, S12 and S20 because the antibodies available do not form stable immunocomplexes during sucrose gradient centrifugation.

An explanation for the inability of antibodies specific for protein S1 to form stable 30S-IgG-30S complexes could be that the antibody removes protein S1 from the ribosome. This explanation would also be supported by our data, since protein S1 is easily removed from the 30S subunit by treatment with LiCl.

For antibodies specific for the other four proteins, the lack of dimerization could be due to different reasons. First, it should be recognized that only a minority of the antibodies raised against an isolated ribosomal protein is reactive with antigenic determinants which are accessible for antibody binding in the intact subunit. Thus it is possible that our antibodies are specific for epitopes which are not exposed in the intact 30S subunit. However, the fact that our antibodies specific for S8, S9, S12 and S20 do not react with intact subunits, does not allow the conclusion that these proteins are buried within the 30S particle. In fact, other authors localized proteins S8 and S12 by antibody label thus showing that antigenic determinants of these two proteins must be accessible at the surface of the subunit (Kahan e al., 1981; Lake, 1980). On the other hand, we were able to localize S4 (Stöffler-Meilicke et al., 1984) which has been claimed by these authors to be inaccessible based on negative antibody label results.

For all these reasons, it was interesting in this study to compare the accessibility of ribosomal proteins on 30S subunits and core particles.

We tested several antibodies which are not able to dimerize 30S subunits with 0.75 M core particles (see 3.6.). With the only exception of anti-S4, none of the IgG preparations reacted with the core particles. On the basis of these results, it seems unlikely that the lack of dimerization is due to the antigenic determinants being inaccessible to antibody binding. In fact, neutron scattering studies suggested that at least protein S12 is located in the upper one-third of the particle, from which a number of proteins are deleted by salt (Ramakrishan et al., 1981). In addition, the fact that proteins S9 and S12 are removed from the core particles when the LiCl concentration is increased to 1.0 M, indicates that these two proteins should be close to the surface of the 0.75 M LiCl core particles. Protein S20 should also be located at the surface of the 30S subunit, since after dissociation of the 70S ribosome, 20% of S20 can be found on the 50S subunit. Another example are proteins S16 and S17. At the beginning of this work, these two proteins had not been localized in our laboratory since all the antibodies available were not reactive with 30S subunits. However, by the screening of a large number of IgG preparations one anti-S16 and one anti-S17 were found which reacted with both core particles and 30S subunits. These two antibodies were used to localize the respective proteins (Stöffler-Meilicke and Stöffler, 1984).

These observations altogether, suggested that reasons other than the antigenic determinants of a protein being not exposed might be responsible for the lack of reactivity of several antibodies. It has been shown that different antibodies react with the antigen with different affinity, the association constants ranging between 4×10^3 and 1×10^9 l/mol (Eisen, 1973). Therefore, pairs of subunits joined by an IgG molecule only survive sucrose gradient centrifugation if the antibody has a high affinity. It is possible that the antibodies we tested bind to the subunits with too low an affinity. Evidence that antibodies against proteins S8, S9, S12 and S20 react

with the 30S subunit although they do not lead to the formation of immunocomplexes in sucrose gradient, was provided by in situ immunospecific neutralization experiments in which it was shown that Fab fragments from these IgGs inhibit specific functions of the 30S subunit (Lelong et al., 1979).

In conclusion, our results indicate that most of the antibodies which do not dimerize 30S subunits are also unreactive with core particles. For this reason, we think we shall better be able to localize the proteins still to be mapped by screening a large number of IgGs.

4.4. Localization of protein S4 on the surface of 30S core particles

Protein S4 has recently been localized by immunoelectron microscopy at the small lobe of 30S subunits of Escherichia coli and Bacillus stearothermophilus; the same location was deduced from the mapping of residue Cys-31, which was haptenated with fluorescein and localized with fluorescein specific antibodies (Stöffler-Meilicke et al., 1984). This study clearly demonstrated that epitopes of protein S4 are accessible for antibody binding on intact small ribosomal subunits.

In contrast to this, Winkelmann and Kahan (1979) reported that three rabbit antisera against S4 reacted with 30S reconstitution intermediates lacking proteins S5 and S12, but not with complete 30S subunits. These authors showed that proteins S5 and S12 together are responsible for masking S4 in the 30S subunit, since the maximal reactivity with anti-S4 was obtained only when both proteins were omitted from the subunit. It was concluded that protein S4 is buried in the complete subunit, although this conclusion applies strictly to particular epitopes of protein S4 and need not apply to the entire pro-

tein.

S4 was localized by immunoelectron microscopy in subribosomal particles lacking S5 and S12 (Winkelmann et al., 1982), again with an antibody preparation that did not react with intact ribosomes (Winkelmann and Kahan, 1983).

For these reasons the location of protein S4 was further investigated on 30S core particles in order to study if more epitopes of S4 are exposed after protein depletion. When the results obtained with core particles are compared with those obtained with 30S subunits, neither increased reactivity in sucrose gradients nor exposure of additional antigenic determinants were detected. The 0.75 M LiCl core particles lack, among other proteins, also protein S5 which has been indicated by Lake and coworkers as responsible for the immunochemical masking of protein S4 in intact 30S subunits. On the other hand it was found that an antibody specific for a fragment of protein S4 did not react with the native subunits but formed immunocomplexes with both 0.75 M and 0.5 M core particles. This result indicates that an additional antigenic determinant of protein S4 is exposed on the surface of the core particles. The core particles obtained by treatment with 0.5 M LiCl are very similar to 30S subunits in their protein composition with proteins S1, S2 and S3 reduced. Proteins S5 and S12 which according to Winkelmann and Kahan, (1979 and 1983) would cover S4 are present in these particles in normal amounts. Therefore it is not clear if the exposure of the epitope in question is due to the depletion of a given protein(s) or rather to conformational changes induced in the core particles.

Even this additional epitope was located on the small lobe, so that we can conclude that our results do not indicate an elongated shape of protein S4.

gated shape of protein S4.

4.5. Are ribosomal proteins elongated?

In early studies using both immunoelectron microscopy (Tischendorf et al., 1975; Lake, 1978) and hydrodynamic and scattering methods (Wong and Paradies, 1974; Rohde et al., 1975; Österberg et al., 1977) several proteins of the 30S subunit were concluded to be highly elongated. Later it was recognized that the multiple antibody binding sites observed in the electron microscope were due to contaminating antibodies and that the hydrodynamic and small-angle-X-ray-scattering measurements were significantly influenced by the procedure used to extract the proteins. X-ray crystallography and neutron scattering studies showed that most proteins are fairly compact within the ribosome. (For a review of the current knowledge on the structure of ribosomal proteins see Giri et al., 1984). However, there is still some debate about the shape of some proteins. For example, although protein S13 has been reported to be somewhat asymmetrical (Giri et al., 1984), it has been crosslinked with 9 other proteins of the 30S subunit; this result strongly suggests an extended conformation of protein S13 within the subunit (Traut et al., 1980). We investigated the occurrence of elongated proteins within the 30S subunit by searching for additional antigenic determinants of S4, S13 and other proteins on the surface of protein depleted core particles (3.5.). However, we did not detect well separated determinants for any of the proteins studied . Therefore our results did not provide evidence that elongated proteins exist in the 30S subunit although they cannot rule out this possibility.

5. RESULTS: TOPOGRAPHY OF THE 50S SUBUNIT

The second topic of my work was to localize ribosomal proteins on the surface of the 50S subunit. When this study was started, only 8 proteins, namely L1, L7/L12, L10, L11, L17, L18 and L19 had been localized by immunoelectron microscopy and preliminary results on the location of protein L25 were available (Noah, 1982). One of the main problems which beset the localization of proteins on the surface of the 50S subunit was that many of the antibodies specific for individual proteins of the 50S subunit do not yield stable immunocomplexes necessary for immunoelectron microscopy.

In the attempt to localize more proteins of the 50S subunit, different strategies were employed:

a) A large number of antibodies specific for the proteins still to be mapped was screened for its reactivity with 50S subunits.

b) All antibodies which were not reactive with complete 50S subunits were tested for reactivity with core particles. This approach was undertaken in order to determine whether these IgG preparations are specific for antigenic determinants which are not accessible on the surface of the intact subunit.

c) The specificity of the antibodies which were judged to be suitable for immunoelectron microscopy was assessed by a new set of control experiments. The IgGs whose specificity could be demonstrated by these controls, were used for the localization of the corresponding proteins on the 50S subunit.

5.1. CORE PARTICLES FROM 50S SUBUNITS

5.1.1. Homogeneity of the 50S core particles as judged by sucrose gradient centrifugation

Core particles were obtained from 50S subunits by treatment with 1.5 M LiCl (see 2.5.).

The comparison of the gradient profiles obtained after sucrose density gradient centrifugation of 50S subunits and 1.5 M LiCl core particles shows that the core particles are lighter than the 50S ribosomes and sediment in a gradient region corresponding to lower sucrose density (Fig.19). The core particles sediment as a single peak only slightly wider than the 50S peak, indicating that they are physically homogeneous.

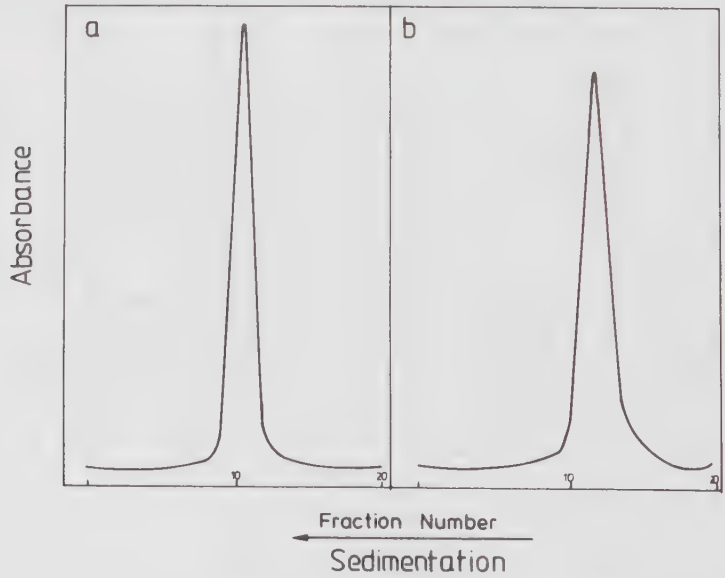


Fig.19. Sucrose density gradient centrifugation of (a) 50S subunits and (b) 1.5 M LiCl core particles.

5.1.2. Protein composition of 1.5 M LiCl core particles

Proteins from the 50S core particles were extracted with acetic acid and analyzed by both cellulose acetate gel electrophoresis and double immunodiffusion.

An electropherogram of TP cores, control TP50 and split proteins is shown in Fig.20. The electropherogram of TP50 from the control subunits displays the usual pattern of ribosomal proteins of the 50S subunit (compare Fig.20, tracks 4 and 5 with Fig.3). In the lanes of TP from core particles the bands corresponding to proteins L7/L12, L9, L10, L11, L15, and L27/L28 are missing (Fig.20, tracks 2 and 3, ►) and the band corresponding to protein L6 appears to be reduced (Fig.20, tracks 2 and 3, →). Also fainter than in the control are the bands where proteins L1/L3/L21/L25, L14/L18/L30 and L2/L16 comigrate (Fig.20, tracks 2 and 3, →). The electropherogram of the split proteins confirmed that proteins L7/L12, L9, L10, L11, L15, L27 and L28 have been removed from the 50S subunits by LiCl treatment (Fig.20, track 1).

In the cases where a band in which more than one ribosomal protein migrate is missing or reduced, a decision cannot be made as to which of the proteins has been removed. This information was provided by immunodiffusion experiments in which total proteins from 50S subunits or from core particles were reacted with antisera specific for individual proteins of the 50S subunit. By this test it was confirmed that L7/L12, L9, L10, L11, L15, L27 and L28 are completely absent from the cores. In addition, it was found that out of the proteins which migrate in the bands of proteins L1/L3/L21/L25, L17/L18/L30 and L2/L6, proteins L1, L3, L16, L25 and L30 are missing, whereas proteins L2, L17, L18 and L21 are contained in the core particles (Table 8). The immunodiffusion test also demonstrated the absence in the core particles of protein

L6 which in the electropherogram appeared to be present in reduced amount.

Thus, 14 proteins are deleted from the 50S subunit by treatment with LiCl, corresponding to 46% of the total protein mass.

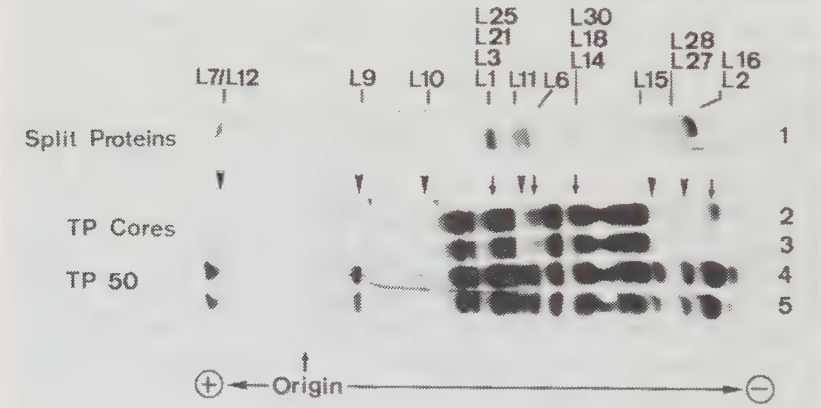


Fig.20. Cellulose acetate gel electrophoresis. Track 1: 50 µg split proteins; tracks 2 and 3: 75 and 50 µg of TP from 1.5 M LiCl core particles; tracks 4 and 5: 75 and 50 µg TP50.

Table 8. Protein composition of 1.5 M LiCl core particles.

PROTEIN	CAG	OUCH	PROTEIN	CAG	OUCH
L1	?	-	L18	?	+
L2	?	+	L19	+	+
L3	?	-	L20	+	?
L4	+	+	L21	?	+
L5	+	+	L22	?	+
L6	red	-	L23	+	+
L7/L12	-	-	L24	+	+
L9	-	-	L25	?	-
L10	-	-	L27	-	-
L11	-	-	L28	-	-
L13	+	+	L29	+	+
L14	?	+	L30	?	-
L15	-	-	L31	+	?
L16	?	-	L32	?	+
L17	+	+	L33	?	-
			L34	?	+

? = not unambiguously determined by this method
CAG = cellulose acetate gel electrophoresis
OUCH = double immunodiffusion

5.1.3. Electron microscopy of 50S core particles

An overall view of 1.5 M LiCl core particles is shown in Fig.21. About 50% of the observed particles display rounded outlines and cannot be ascribed to either the crown or the kidney view, since they completely lack distinctive features. Out of the interpretable forms seen in the core preparation, the majority are observed in the crown projection. The dimensions of the core particles are similar to those observed

for 50S particles.

The analysis of selected images seen at a higher magnification occasionally allows a discrimination of the two side protuberances (Fig.21b). In some cases, a stalk like structure can be recognized (Fig.21b, arrowheads on frames 1 and 2). In other particles, the L1 protuberance can be identified because of its characteristic features, such as the cleft between the central and the L1 protuberances (Fig.21b, arrowhead on frame 4). Other crown forms do not allow a discrimination of the two lateral protuberances; in these particles, only the central protuberance can be identified. However, the central protuberance may be reduced in size (Fig.21b, frame 5). Indentations seen in the middle at the base of the particles (see arrows in Fig.21b) have been also observed in 50S subunits (Noah, 1982) and are therefore not characteristic features of the core particles.

From these observations it can be concluded that the 50S core particles retain their structural compactness; however, they are not suitable for the localization of proteins by immunoelectron microscopy, since most of the particles lack distinctive landmarks providing the points of orientation which are necessary to determine the antibody binding site.

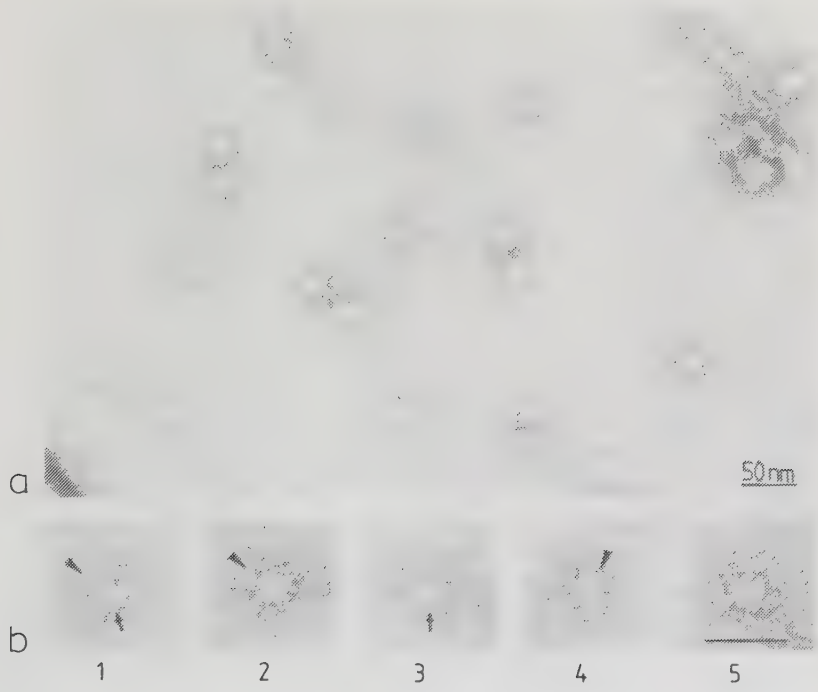


Fig.21. Electron micrographs of 1.5 M LiCl core particles. (a) Overall view; (b) selected particles. For details see text.

5.1.4. Sucrose density gradient centrifugations of 1.5 M LiCl core particles with antibodies specific for 50S proteins

Several IgG preparations specific for proteins contained in the core particles and which were unreactive with 50S subunits were tested in sucrose gradients for their reactivity with the core particles. This survey was undertaken in order to investigate whether these antibodies were specific for antigenic determinants which are not accessible on the surface of the intact 50S subunit. The results are shown in Table 9. Out of

Table 3. Reactivity of 1.5 M LiCl core particles with IgGs which do not dimerize 50S subunits

Antibody to protein	Antibody preparation	A_{280}	Dimer formation 50S	cores
L2	R1020 Pool ACG DEAE cut 3	3.0	-	25%
L2	H171 IgG	3.0	-	-
L2	H14 DEAE cut 1/2 ex SPL2	3.0	-	-
L3	R1021 IgGA	2.5	-	-
L3	H182 IgG	3.0	5%	5%
L4	H12 ACG TP70,TP50	0.8	-	27%
L4	R1165 IgGA	2.5	-	-
L5	H141 DEAE cut 1/2 ex ACG TP50	2.5	-	-
L13	R934 IgGA	3.0	-	-
L13	H119 ACG TP50	0.8	-	-
L14	H117 ACG TP50	1.0	-	-
L21	H157 ACG Pool	2.0	-	-
L22	H162 ACG TP50	1.0	-	-
L22	R1128 IgGA	3.0	5%	5%
L24	R952 IgGA	3.0	-	-
L24	R951 IgGA	3.0	-	-
L32	H94 DEAE cut 1/2	3.0	-	-
L32	H175 IgG	3.0	-	-

the antibodies tested, only two, namely the anti-L2 from rabbit R1020 and the anti-L4 from sheep H12, yielded ribosome-antibody complexes when reacted with 1.5 M LiCl core particles. Unfortunately, it was not possible to demonstrate the specifity of these two IgG preparations using the standard absorption experiments; this result does, however, demonstrate that the removal of proteins by LiCl can lead to the exposure of determinants which in the intact 50S subunit are not accessible.

Since I was not able to use the two IgG preparations (which were reactive with 50S cores but not with intact 50S particles) for the localization of the respective proteins, 50S core particles were used as a quick method to screen antibody preparations raised against individual proteins which are reactive with 50S subunits. The core particles lack a defined set of proteins and therefore they provide a simple test to exclude a priori the occurrence of certain contaminants in the IgG preparation. In the first set of experiments, the core particles were incubated with antibodies specific for split proteins (Table 10). Anti-L9 and anti-L27 did not react with core particles (Table 10). This result, although not sufficient to demonstrate the specificity of the antibody, allowed us to exclude the presence of contaminating antibodies directed against any of the split proteins. The specificities of the two antibodies for proteins L9 and L27, respectively, were further assessed by the standard controls and they were therefore used to localize the two proteins by immunoelectron microscopy (see 5.3. and 5.8.). In contrast, anti-L25 dimerized the core particles although they do not contain protein L25 (see Table 8); in this case we concluded that the formation of immunocomplexes was due to contaminating antibodies specific for proteins other than L25.

Table 10. Reactivity of 1.5 M LiCl core particles with IgG specific for split proteins.

Ab to protein	Antibody prep.	A_{280}	Dimer formation 50S	cores
L9	R1046 IgGA	1.5	38%	-
L25	H115 ACG Pool	0.5	15%	15%
L27	R944 ACG cut3	0.6	20%	-

In another set of experiments, the core particles were reacted with antibodies specific for core proteins and which were reactive with complete 50S subunits (Table 11). Three IgG preparations, anti-L3 R1021, anti-L14 R294 and anti-L22 R1161 did not dimerize the core particles, thus showing that the formation of 50S-immunocomplexes was due to contaminating antibodies reactive with one or more of the split proteins. All other antibodies reacted with both 50S subunits and with core particles. In these cases, the presence of contaminating antibodies directed against any of the proteins which are removed by LiCl could be excluded. Further experiments were undertaken to assess their specificity for the respective ribosomal proteins and showed the suitability of the anti-L4 from rabbit H172 for the localization of protein L4 (see 5.5.).

Table 11. Reactivity of 1.5 M LiCl core particles and 50S subunits with antibodies specific for "core" proteins

Antibody to protein	Antibody preparation	A_{280}	Dimer formation 50S	cores
L3	R1021 ACG TP50,TP70 cut B	0.2	40%	-
L14	R294 ACG TP70 cut B	1.0	15%	-
L22	R1161 IgGA	2.0	15%	-
L3	RPool ACG TP50,TP70 cut B	1.0	35%	30%
L4	H172 ACG TP50,TP70 cut B	2.0	15%	15%
L4	R178 DEAE cut 1/2	1.0	12%	12%
L4	RPool ACG TP70 cut 3	0.5	21%	20%
L14	R229 IgGA ex Eluat TP70 cut 3	3.0	20%	18%
L20	RPool IgGA	3.0	15%	17%
L20	H185 DEAE cut 1/2	3.0	13%	12%
L21	R392 IgG	3.0	12%	12%
L22	R234 IgGA	1.5	15%	10%
L22	RPool ACG TP70 cut 3	1.0	25%	20%

5.2. Localization of ribosomal proteins on the surface of the 50S subunit

Altogether six proteins of the 50S subunit, namely L4, L5, L6, L9, L15 and L27 have been localized by immunoelectron microscopy. The antibody preparations available which are specific for these six proteins are listed in Table 12.

The specificity of all antibody preparations used in this work was determined by the following controls which have been recently developed in our laboratory (see also Stöffler and Stöffler-Meilicke, 1984):

(1) The formation of immunocomplexes must be inhibited if the antibody is preincubated with the purified antigen protein. This experiment allows one to demonstrate that the determinant to which the antibody binds is contained in the protein to be localized. However, this test alone is not sufficient to exclude cross-reaction of the antibody with antigenic determinants on other ribosomal proteins. This possibility must be considered since the minimal size of an antigenic determinant is a tetrapeptide (Sela, 1969) and common oligopeptides among ribosomal proteins have been found with a high frequency (Wittmann, 1982).

(2) The occurrence of crossreacting antibodies can be excluded if the ability of the antibody to dimerize the ribosomes is not inhibited by preincubation with a mixture of all ribosomal proteins lacking only the protein in study. Such protein mixtures can be obtained in different ways. One possibility is to extract proteins from mutant ribosomes which lack a single ribosomal protein. This kind of control experiment was first performed in a study carried out to localize protein L1 (Dabbs et al., 1981). Since this time, several mutants lacking one ribosomal protein have been isolated (Dabbs et al., 1983). Protein mixtures from mutant ribosomes have been used to assess the specificity of anti-L6 (5.4.2.) and anti-L27

(5.8.2.). If mutant ribosomes are not available, mixtures lacking a single ribosomal protein can be prepared by mixing purified proteins in stoichiometric amounts. An alternative strategy was developed to test the purity of anti-L4 (5.5.2.) and anti-L5 (5.7.2.) by passing total ribosomal proteins over a Protein A-Sepharose column to which IgG from rabbit directed against the protein to be removed had been bound (see 2.14.1.).

Finally, the protein can be localized by electron microscopy. For this purpose, the antibody binding site must be determined on both projectional forms of the 50S subunit, the crown form and the kidney form. Immunocomplexes in which a crown view is combined with a kidney view through a single IgG molecule demonstrate the identity of the epitopes on the two views and allow a three-dimensional localization of the protein on the 50S subunit.

Table 12. Antibodies specific for individual proteins of the 50S subunit.

Protein	Antibody	Reactivity	Specificity
L9	*R1046 IgG	+	+
	R1045 IgGA	+	n.d.
	H153 ACG TP50	-	
L6	*R1074 IgGA	+	+
	H78 IgG	-	
	H168 ACG cut B	-	
L4	*H172 ACG	+	+
	TP50,TP70 cut B		
	H12 ACG TP50,TP70	-	
	R1165 IgGA	-	
	R178 DEAE 1/2	+	n.d.
	RPool ACG		
L15	*R1049 IgGA	+	+
	R1048 IgGA	+	n.d.
	H112 DEAE 1/2	-	
L5	*R1081 IgGA	+	+
	*H142 ACG TP70 cut B	+	+
	H141 ACG	-	
L27	*R944 ACG cut3	+	+
	R238 IgGA	+	-

* Antibody used to localize the correspondent protein

5.3. Protein L9

5.3.1. Reactivity of 50S subunits with anti-L9

For the localization of protein L9, IgG from three antisera (two from rabbit and one from sheep) were available, whose specificity for protein L9 had been assessed by the standard immunochemical techniques. In sucrose gradients, the IgG from sheep 153 was not reactive with 50S subunits, while the two IgGs from rabbit formed stable 50S-IgG-50S complexes (Table 12). For all further experiments IgG from rabbit R1046 was used since it was the most reactive. Maximally 50% of the 50S subunits dimerize at high antibody concentration (Fig. 22a).

5.3.2. Antibody specificity for protein L9

The anti-L9 preparation used did not form dimers with 50S core particles (see Table 10). This experiment provided preliminary evidence for the specificity of the antibody since protein L9 is not present in cores. In order to demonstrate that the reactive epitope on the surface of the large subunit is provided by protein L9, we investigated the inhibitory effect of L9 upon dimer formation. For this experiment we chose an antibody concentration which dimerizes 40% of the subunits. Since protein L9 is present at one copy per ribosome, 2.0 A_{260} of 50S subunits contain 86 pmol of L9 (MW of the 50S subunit = 1.45×10^6 daltons). Preincubation of anti-L9 with 50 pmol of purified protein L9 already has an inhibitory effect upon dimer formation. Preincubation with 300 pmol completely abolished the formation of immunocomplexes (Fig. 22b). Absorption with TP70 also inhibits dimer formation (Fig. 22b).

In contrast, preabsorption of the antibody with a protein mixture which contains all 50S proteins except protein L9, has no effect upon dimerization (Fig. 22b). The protein mixture lacking protein L9 had been prepared by mixing single ribosomal proteins in equimolar amounts.

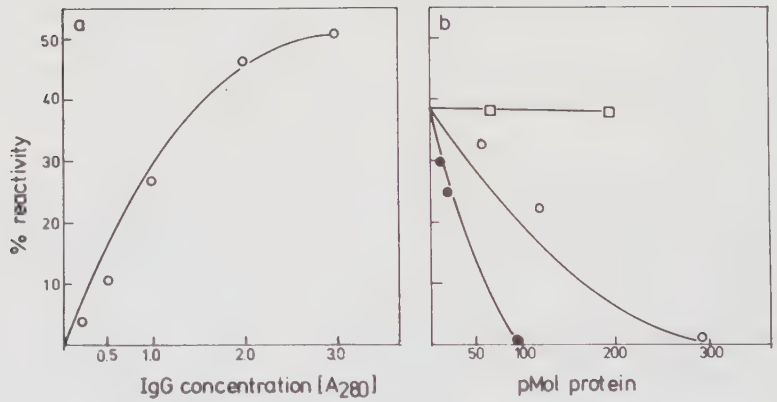


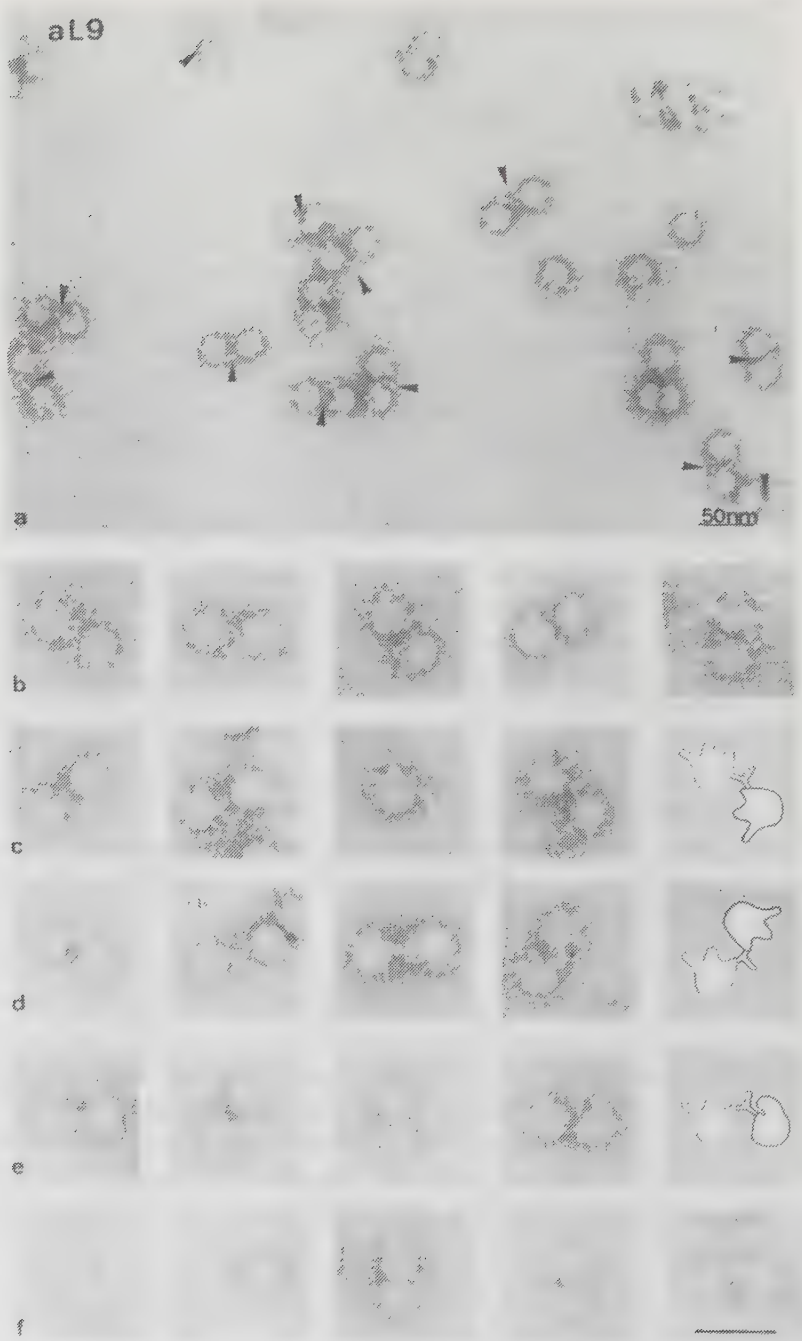
Fig.22. Sucrose gradient centrifugation with anti-L9. (a) Reactivity of anti-L9 R1046 with 50S subunits plotted against antibody concentration. (b) Inhibition of dimer formation by preincubation of the antibody with increasing amounts of single protein L9 (○–○), TP70 (●–●), TP70 lacking protein L9 (□–□).

5.3.3. Localization of protein L9 by immunoelectron microscopy

A field of 50S subunits reacted with anti-L9 and negatively contrasted with 2.5% uranyl formate is shown in Fig. 23a. Typical immunocomplexes are indicated by arrows. About 75% of the subunits are present as dimeric immunocomplexes connected by either one or two IgG molecules. Monomeric immunocomplexes are rare (2%). Pairs of subunits joined by two antibodies account for 25% of the dimers (Fig23b). This observation implies that at least two epitopes of protein L9 are exposed on the surface of the large subunit. Because the Fab arm of an IgG molecule is 3.5 nm in diameter, the two epitopes must be at least 3.5 nm apart.

Immunocomplexes are shown at higher magnification in Fig. 23b-f. In 82% of the complexes the two characteristic views of the 50S particle can be identified. In some cases the subnits are seen in unusual projectional forms (Fig. 23f). In the crown view the antibody attaches in the region of the L1 protuberance (Fig. 23b-d). We observed apparent antibody binding sites at or below the protuberance. Close inspection of the immunocomplexes revealed that the antibody attachment site was between the top of the L1 protuberance (Fig. 23d) and the base of the particle (Fig. 23c). If antibody binding was at the L1 protuberance, the Fab arm of the connecting antibody was only partly visible.

Fig.23. Electron micrographs of 50S subunits reacted with anti-L9. (a) General field with characteristic immunocomplexes indicated by arrows. (b-e) Selected immunocomplexes in which the subunits are seen in the crown projection (b-d). In (e) a crown and a kidney view are connected by the same IgG molecule. (f) Pairs of subunits seen in rare projectional forms. Each scheme illustrates the micrographs to its immediate left.


aL9
a
50nm
b
c
d
e
f

In the kidney view, the antibody binds at the bulge between the notch and the blunted end, on the side that in the 70S monosome faces the 30S subunit (Fig. 23e). Immunocomplexes in which a crown view is connected with a kidney view by one and the same antibody demonstrate that the two antibody binding sites correspond to one and the same epitope in the three-dimensional structure (Fig. 23e). As shown in Fig. 23, aberrant binding sites were not detected.

These results are consistent with a localization of L9 at a single three-dimensional site on the base of the L1 protuberance (Fig. 24).

Fig. 23f represents dimers in which either one or both subunits are seen in particular projectional forms that usually are not observed in preparations of isolated 50S. Such forms may represent views of the 50S subunit standing on end, i.e., adsorbed to the support film by either its central protuberance or its base. These projectional forms are also consistent with a location of L9 at the base of the L1 protuberance.

Fig.24. The location of protein L9 on the three-dimensional model of the 50S subunit.

5.3.4. Accessibility of protein L9 in the 70S ribosome

When reacted with 70S ribosomes anti-L9 forms immunocomplexes, thus showing that the reactive epitope of L9 is not covered by the 30S subunit after association of the two subunits (Fig.25).

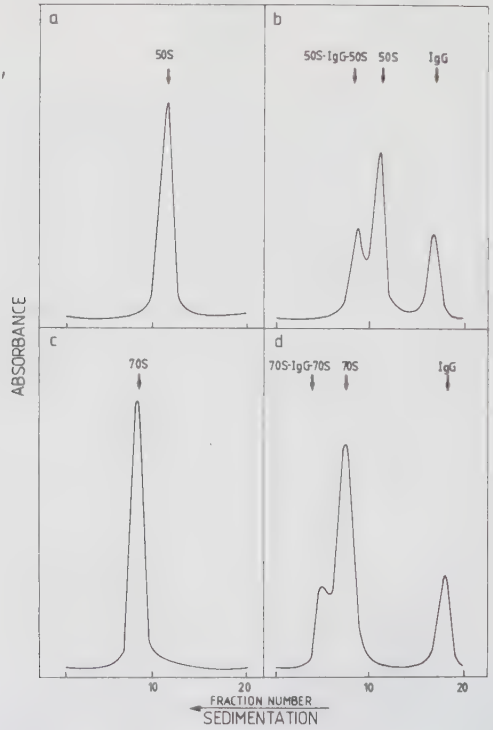


Fig.25. Gradient profiles of 50S (a) and 70S ribosomes (c), alone or reacted with anti-L9 (b) and (d).

5.4. Protein L6

5.4.1. Reactivity of 50S subunits with anti-L6

Three L6-specific IgGs were tested for the formation of immunocomplexes with 50S subunits by sucrose gradient centrifugation (Table 12 and Fig.26a). Both IgGs from sheep were unreactive, while the IgG from rabbit 1074 formed stable 50S-IgG-50S complexes. Already with 1.0 A_{280} of IgG 60% of the subunits are dimerized (Fig.26a). At higher antibody concentrations material appears that sediments slightly faster than the 50S-IgG-50S complexes, probably containing aggregates which consist of more than two subunits linked by antibodies(Fig.26a). This observation suggests that more than one epitope of protein L6 should be exposed at the surface of the large subunit. At a concentration of 3.0 A_{280} units of antibody, approximately 90% of the subunits are reactive, sedimenting as 50S-IgG-50S complexes or faster (Fig.26a).

5.4.2. Antibody specificity for protein L6

The specificity controls were performed using 0.7 A_{280} of IgG R1074 which dimerizes about 30% of the subunits. The formation of immunocomplexes is completely inhibited by 10 µg of purified protein L6 (Fig.26b). The protein had been isolated by a mild extraction procedure which maintains the ribosomal proteins in their native conformation (Dijk and Littlechild, 1979) and had been also used as immunogen for rabbit 1074. On the contrary, various preparations of purified protein L6 isolated by a procedure that implies the use of urea in the buffer, had only little effect upon dimer formation. Since L6 is frequently contaminated with proteins L7/L12, we wanted to rule out that the reaction of the antibody was due to the presence of IgG specific for proteins L7/L12. Therefore the IgG

was preincubated with purified proteins L7/L12: no effect was observed upon dimerization (not shown). The specificity of the antibody for protein L6 was unambiguously demonstrated by preabsorption with total ribosomal proteins which lack only protein L6, obtained from mutant R15-22: the formation of immunocomplexes was not inhibited (Fig.26b). In contrast, dimer formation is completely inhibited by preabsorption with TP70 from wild type (Fig.26b).

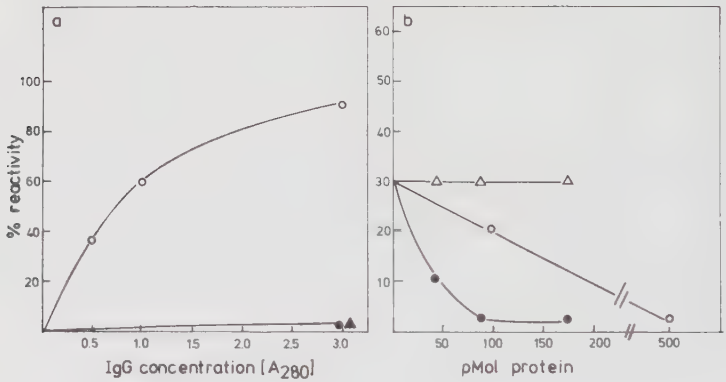


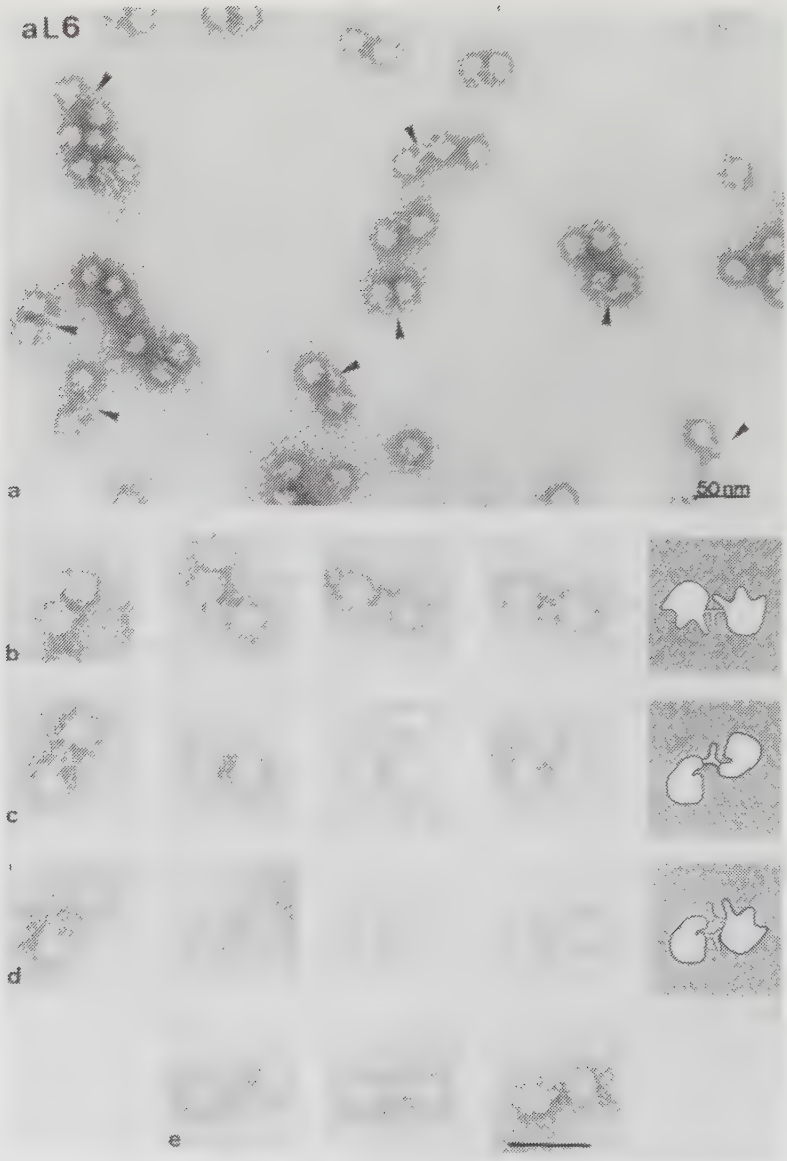
Fig.26. Sucrose density gradient centrifugation with anti-L6. (a) Reactivity of 50S subunits with IgG from R1074 (o——o), H78 (Δ—Δ), H168 (●—●). (b) Inhibition of antibody-ribosome complex formation by preincubation of anti-L6 R1074 with increasing amounts of purified protein L6 (o—o), TP70 (●—●), TP70 lacking protein L6 (Δ—Δ)

5.4.3. Localization of protein L6 by immunoelectron microscopy

The general field of 50S subunits reacted with anti-L6 shows 86% of the subunits to be present as IgG-linked pairs and only 1% as monomeric 50S-IgG complexes (Fig.27a). 30% of the evaluated dimers are simultaneously connected by two antibody molecules. In the immunocomplexes the subunits are observed in both the crown and the kidney view, the kidney form being relatively frequent. In approximately 20% of the dimers the orientation of the subunits and the antibody binding site cannot be interpreted. Noticeable amounts of unbound IgG molecules are visible on the support film (Fig.27a).

A gallery of individual micrographs is shown in Fig.27b-e. In the crown form the antibody attaches at the base of the L7/L12 stalk (Fig.27b). The Fab arms of the linking IgG are fully visible in most dimers. Thus, the antibody binding site must be close to the contours of the subunit. In the kidney view, the antibody binds to the flat side of the subunit, between the pointed end and the notch (Fig.27c). Less than 2% of the immunocomplexes show antibody binding at sites other than those described.

Fig.27. Electron micrographs of 50S subunits reacted with anti-L6 (a) General view, (b-e) selected immunocomplexes in which the subunits are seen in the crown view (b), in the kidney view (c) and in rare projectional forms (e). (d) Immunocomplexes in which a crown form and a kidney form are connected by the same IgG molecule.


aL6
a
50nm
b
c
d
e

The location of protein L6 on the three-dimensional model of the large subunit is illustrated in Fig.28. L6 is situated at the body of the subunit in the region where the stalk originates. The area dashed on the model covers a relatively large region of the subunit, according to the multiple antibody binding sites observed in the electron micrographs.

Fig.28. The location of protein L6 on the three-dimensional model of the 50S subunit.

Occasionally, dimers are seen with the subunits in projections which are not or are only rarely observed in standard 50S preparations. The same projections have been observed in dimers linked by anti-L11, an antibody which binds in the same region (Noah, 1982) and by anti-L9 which binds at the base of the L1 protuberance (5.3.3.).

The reactive epitope of protein L6 is accessible to antibody binding also in the 70S ribosome (Fig.29).

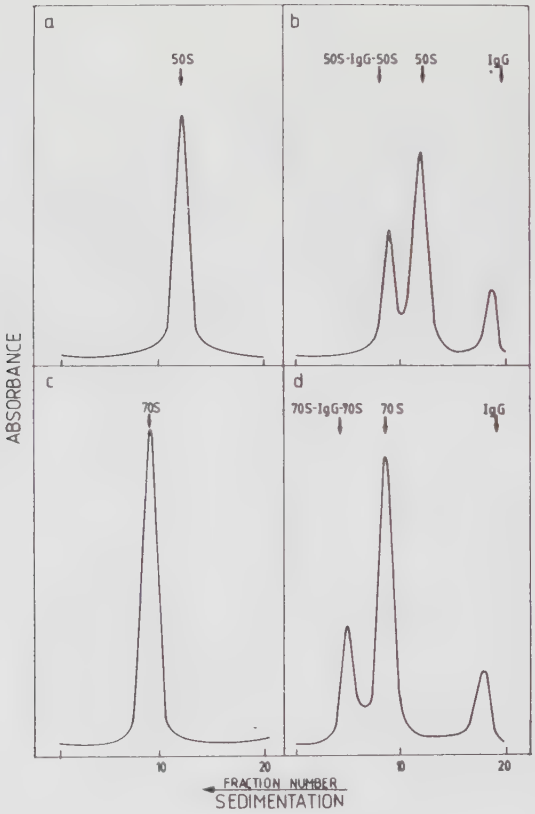


Fig.29. Sucrose gradient profiles of 50S subunits and 70S ribosomes alone (a and c, respectively) or reacted with anti-L6 (b and d).

5.5. Protein L4

5.5.1. Reactivity of 50S subunits with anti-L4

IgG from four antisera were tested in sucrose gradients for their reactivity with 50S subunits. One IgG from rabbit and one from sheep did not form ribosome-antibody dimers. The remaining antibodies were reactive (Table 12). However, they yielded immunocomplexes in very small amounts (10% dimers). In order to obtain a more reactive IgG fraction, IgG was isolated from the serum H172 by affinity chromatography. In spite of this, a maximum of 15% of the subunits react with 2.0 A_{280} of this antibody (Fig.30a). The low reactivity of the antibody with 50S subunits could be due to a low affinity of the antibody but one should also consider the possibility that epitopes of protein L4 are exposed on the surface of a small fraction of 50S subunits only.

5.5.2. Antibody specificity for protein L4

Anti-L4 was preincubated with increasing amounts of purified protein L4, which had been extracted under non denaturating conditions (Dijk and Littlechild, 1979). The amount of dimerized subunits decreases with increasing amounts of protein (Fig.30b). At a concentration of 10 µg (450 pmol) of protein or more, 5% of the subunits are still dimerized (Fig.30b). In contrast, addition of TP70 completely abolishes the formation of dimers (Fig.30b). When the antibody is incubated with TP50 lacking protein L4, the dimerization is reduced by about 4% (Fig.30b). The protein mixture which lacks protein L4 was prepared by immunoaffinity chromatography (see 2.14.1.). The immunoelectrophoresis shown in Fig.31 demonstrates that protein L4 is removed from the protein mixture.

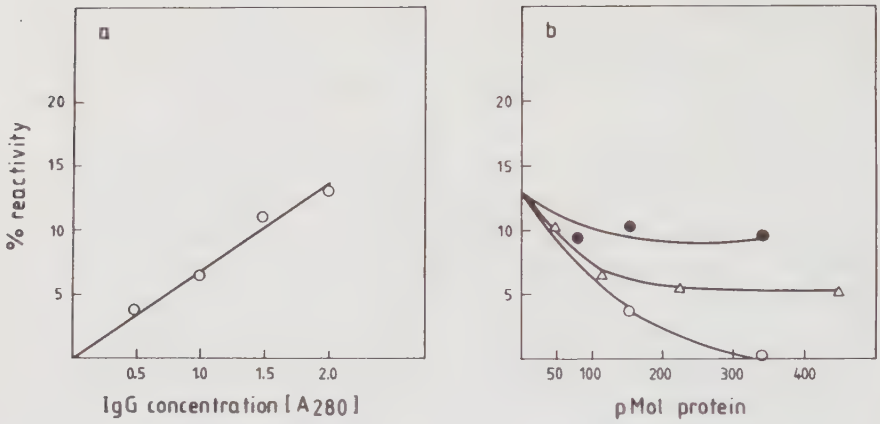


Fig.30. Sucrose gradient centrifugation with anti-L4. (a) Reactivity of 50S subunits with anti-L4. (b) Inhibition of dimer formation by incubation of 2.0 A_{280} of anti-L4 with increasing amounts of purified protein L4 (Δ–Δ), TP70 (o–o), TP50 lacking protein L4 (●–●).

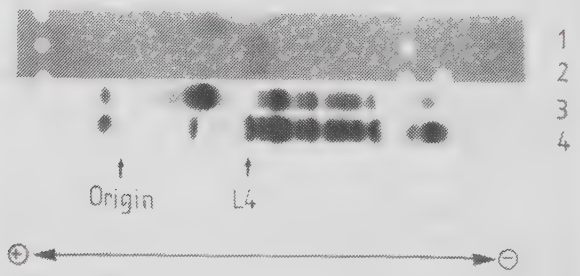


Fig.31. Cellulose acetate gel electrophoresis and immunoelectrophoresis. Tracks 1 and 3: 100 µg TP50 lacking protein L4; tracks 2 and 4: 100 µg TP50. After electrophoresis, tracks 3 and 4 were stained directly, tracks 1 and 2 were washed with PBS and then soaked overnight in anti-L4 (see 2.12.).

The experiments reported above suggest the presence of a small fraction of contaminating antibodies in the antibody preparation specific for protein L4. Preincubation of the antibody with TP50 lacking protein L4 eliminates contaminating antibodies. Therefore, the dimer peak obtained after preabsorption with TP50 lacking L4 was used for the localization of L4 by immunoelectron microscopy.

5.5.3. Electron microscopy of 50S-anti L4 complexes

When immunoelectron microscopy was performed on immunocomplexes obtained by reacting 50S subunits with anti-L4 which had not been preabsorbed with TP50 lacking L4, we observed antibody binding to the central protuberance in 20% of the dimers, in addition to the binding sites characteristic for anti-L4 (see below). Minor binding sites at the stalk were also observed (results not shown). These binding sites were completely abolished after preabsorption with TP50 lacking L4 (see Fig.32) and are therefore due to contaminating antibodies. When anti-L4 was preabsorbed with purified protein L4, a small fraction of immunocomplexes was still formed (Fig.30b). Electron micrographs of these complexes also showed antibody binding sites at the central protuberance and at the stalk (not shown). The electron microscopic observations confirmed the result of the absorption experiments that the anti-L4 preparation contained a minor fraction of contaminating antibodies.

Contaminating IgG was eliminated by preabsorption with TP50 lacking protein L4 and the dimers formed by the purified antibody were used for the localization of protein L4. Fig.32a shows a general field of 50S subunits reacted with anti-L4. Typical ribosome-antibody complexes are indicated by arrows. 79% of the subunits seen in the electron micrographs are present as dimeric immunocomplexes connected by either one or by

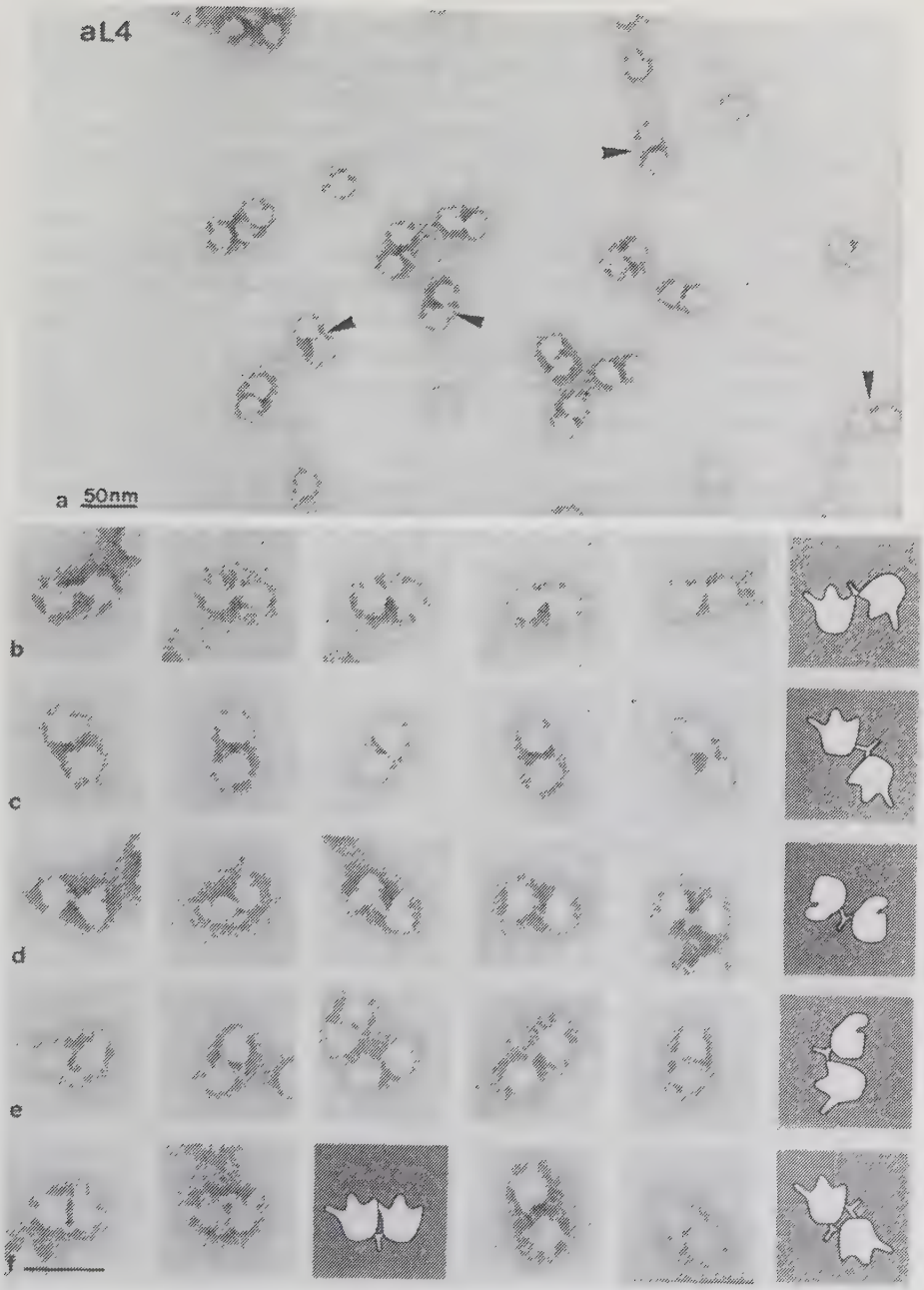
a pair of IgG molecules. Only 1% are present as 50S-IgG complexes. Unbound IgG molecules are frequently observed.

Both projectional forms are seen in the dimers. An accurate positioning of the antibody binding site was possible for about 80% of the evaluated immunocomplexes. When the subunits are seen in the crown form the antibody binds to the side of the wider lateral protuberance (Fig.32b,c). In the dimers shown in row b, the L1 protuberances of both subunits are touching each other and the antibody molecule cannot be seen. In the dimers presented in row c, the IgG can be clearly discerned, although the Fab arms are not seen in their full length. In these images, antibody attachment is observed on the base of the subunit, below the L1 protuberance. Taking into account the length of the Fab arm of the IgG molecule, both observed contact sites are consistent with an actual antibody binding site in the middle region of the subunit.

In the kidney view, the antibody contacts the subunits on the convex side in a region stretching from opposite the notch to the base of the subunit (Fig.32: compare the last frame of row d with the first of row e).

The identity of the epitopes on different views is demonstrated by complexes in which a crown form is connected with a kidney form (Fig.32e). Only 2% of the immunocomplexes showed aberrant antibody binding.

Fig.32. Electron micrographs of 50S subunits reacted with anti-L4. (a) General view, (b-f) selected micrographs. The subunits in (b) and (c) are in the crown projection and those in (d) in the kidney projection. (e) Dimeric immunocomplexes in which a kidney form is connected to a crown form by the same IgG molecule. (f) Dimers connected by two IgG molecules.


aL4
a 50nm
b
c
d
e
f

Examples of dimers simultaneously joined by two IgG molecules are shown in Fig.32f. In the first two frames only one IgG molecule can be clearly seen because the two side protuberances overlap. The occurrence of two antibodies is obvious in the two last frames. In these dimers the two antibody binding sites appear to be quite distant from one another.

These electron microscopic observations are consistent with L4 being situated in the middle of the back of the subunit on the side which faces the L1 protuberance (Fig.33). The large area dashed on the three-dimensional model takes into account the occurrence of at least two epitopes of protein L4 on the surface of the subunit.

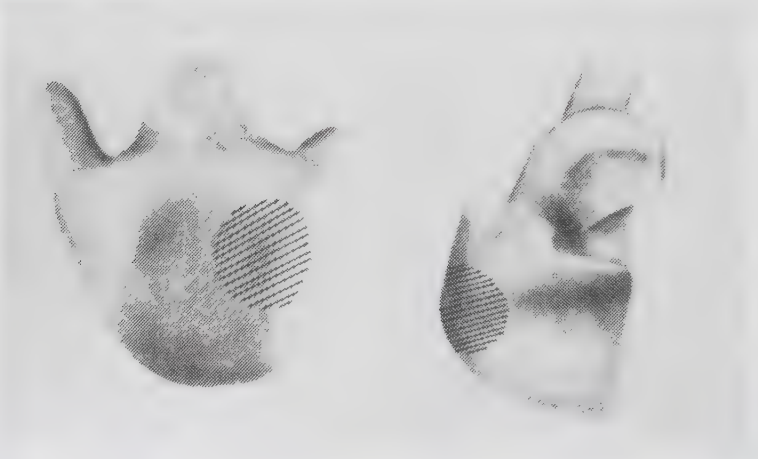


Fig.33. The location of protein L4 on the three-dimensional model of the 50S subunit.

5.6. Protein L15

5.6.1. Reactivity of 50S subunits with anti-L15

For the localization of protein L15 two antisera raised in rabbit were used (Table 12). Another anti-L15 raised in sheep did not react with 50S subunits (Table 12). When IgG preparations from the two antisera were tested for the formation of stable 50S-immunocomplexes, both antibodies reacted strongly with 50S subunits, as judged from sucrose gradient centrifugation (Fig.34a). A maximum of 65% of the subunits reacted with the L15 specific preparations. Since the dimer formation occurs under non equilibrium conditions, it is likely that even a larger part of the subunit population is reactive with anti-L15. It can be concluded that epitopes of protein L15 are exposed on the surface of the majority of the subunits.

IgG from rabbit R1049 was used for all further experiments.

5.6.2. Antibody specificity for protein L15

The formation of dimeric immunocomplexes is completely abolished by preabsorption of anti-L15 with 2 µg of purified protein L15 and with TP70 from wild-type (Fig.34b). On the contrary, preabsorption with TP70 from mutant AM 16-98 which lacks protein L15 (Lotti et al., 1983) does not inhibit dimer formation (Fig.34b). Accordingly, the anti-L15 does not react with 50S subunits from the mutant (Fig.34a).

The results presented in this chapter have been published in Lotti et al. (1983)

Since the formation of immunocomplexes was exclusively dependent on L15-specific antibodies, we used this antibody to determine the location of protein L15 on the surface of the 50S subunit.

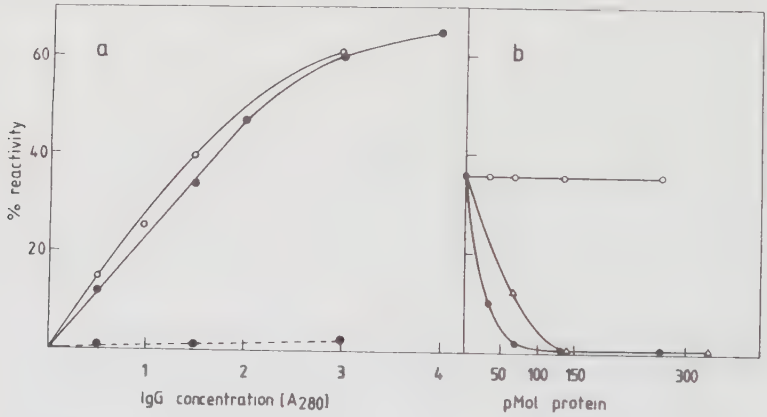


Fig.34. Sucrose gradient centrifugation of anti-L15. (a) Reactivity of 50S subunits from WT (——) and mutant AM 16-98 (- -) with IgG from rabbit 1049 (●—●) or 1048 (o—o), plotted against antibody concentration. (b) Inhibition of ribosome-antibody complex formation by preincubation of the antibody with increasing amounts of purified protein L15 (Δ—Δ), TP70 (●—●), TP70 from mutant AM 16-98 (o—o).

5.6.3. Localization of L15 by immunoelectron microscopy

Fig.35a shows a general field of 50S subunits reacted with anti-L15. Approximately 60% of the subunits are present as dimeric immunocomplexes; monomeric immunocomplexes are also observed. Many of the dimeric immunocomplexes are simultaneously connected by pairs of IgG molecules, which obscure the exact location of the antibody binding site.

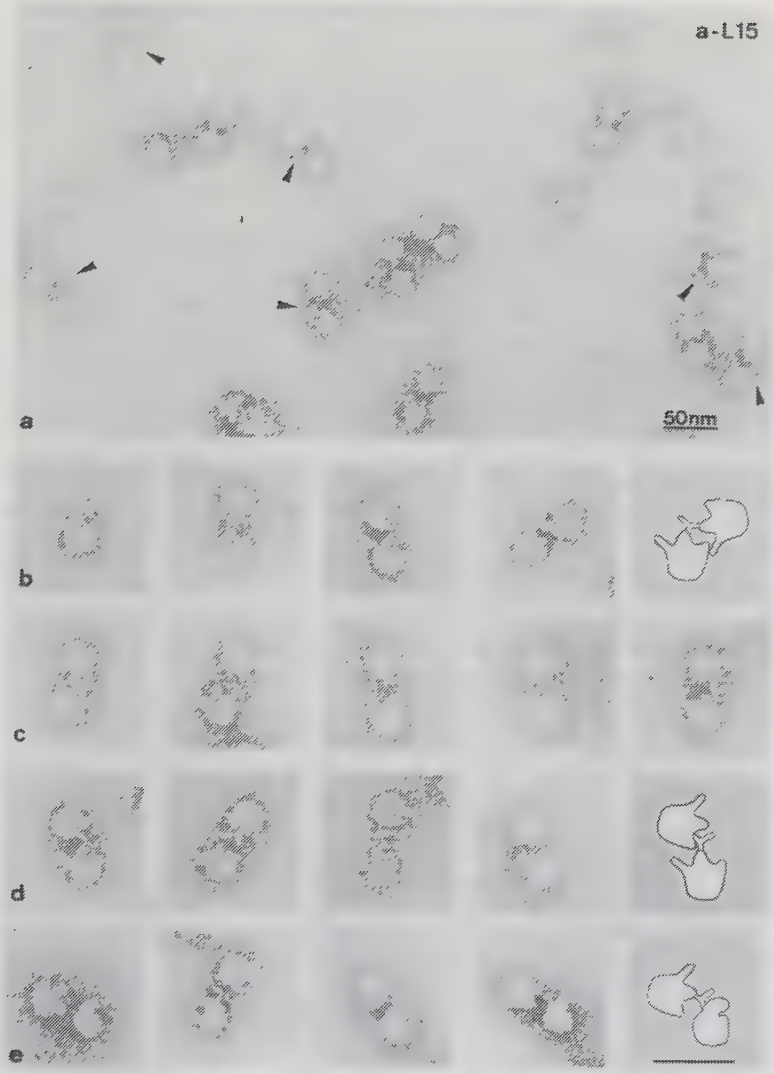


Fig.35. Electron micrographs of 50S subunit reacted with anti-L15. (a) General view; characteristic immunocomplexes are arrowed. (b-e) Selected electron micrographs. The 50S subunits in rows (b-d) are displayed in the crown projection. In the immunocomplexes shown in (e), a kidney projection is connected by one and the same IgG molecule with a crown projection.

Altogether, 386 dimeric and 22 monomeric immunocomplexes have been evaluated, a selection of which is shown in Fig.35b-e. In the crown view, antibodies are attached in the region between the central protuberance and the L1 protuberance (Fig.35b-d). Generally, the Fab arm of the connecting IgG molecule is only partly visible.

In the kidney projection, which is rarely observed, antibody binding is on the convex side of the particle, near to the pointed end (Fig.35e). In the latter immunocomplexes, a kidney view is connected with a crown view, thus demonstrating that the epitopes on the two views are identical. Antibody binding sites other than those described have not been observed.

Electron micrographs of 50S subunits reacted with anti-L15 from rabbit 1048 were consistent with the results described for R1049.

From these observations, protein L15 can be localized on the three-dimensional model at the angle formed between the central and the L1 protuberance (Fig.36).

Fig.36. The location of protein L15 on the three-dimensional model of the 50S subunit.

5.6.4. Accessibility of protein L15 in the 70S ribosome

Next we tested the reactivity of anti-L15 with 70S ribosomes. As shown in Fig.37 anti-L15 reacts with 70S monosomes equally well as with 50S subunits. This result is in good agreement with the finding that in the kidney view antibody binding was observed on the convex side of the particle.

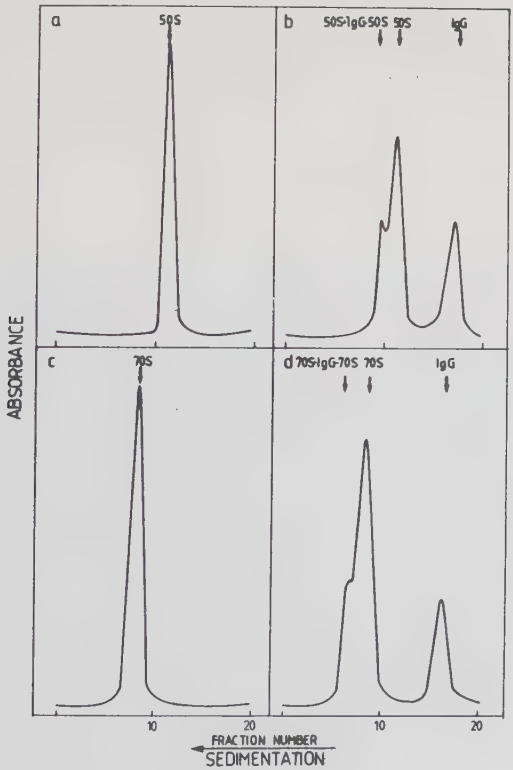


Fig.37. Gradient profiles of 50S subunits and 70S ribosomes alone (a and c) or reacted with anti-L15 (b and d).

5.7. Protein L5

5.7.1. Reactivity of 50S subunits with anti-L5

IgG from three antisera (one from rabbit and two from sheep) whose specificity had been assessed by the standard immunochemical techniques, were tested for their reactivity with 50S subunits by sucrose gradient centrifugation. IgG from one of the sheep was not reactive, while the other antibody preparations formed stable 50S-IgG-50S complexes (Table 12). Maximally 45% of the subunits were reactive with anti-L5 (Fig.38a).

5.7.2. Antibody specificity for protein L5

The dependence of antibody binding on the presence of protein L5 in the large subunit was shown by preincubation of anti-L5 with purified protein L5. 100 pmol of protein L5 completely abolish dimer formation (Fig.38b). Since 2.0 A_{260} of 50S subunits contain 86 pmol of protein L5, it can be concluded that the dimerization is inhibited by purified protein L5 in nearly equimolar amount. Preincubation with TP70 has the same effect (Fig.38b). In contrast, no inhibition of the dimerization is observed if the antibody is absorbed with a mixture of all 50S ribosomal proteins which lacks only protein L5 (Fig.38b). The protein mixture lacking L5 was prepared by passing TP50 through a column to which anti-L5 antibodies had been bound (see 2.14.1.). The absence of L5 in this mixture was demonstrated by double immunodiffusion (Fig.39).

The same set of experiments has also been carried out with IgG from H142: the specificity of the antibody preparation could be shown as for IgG R1081. Therefore, the electron microscopic investigation was performed with both antibodies.

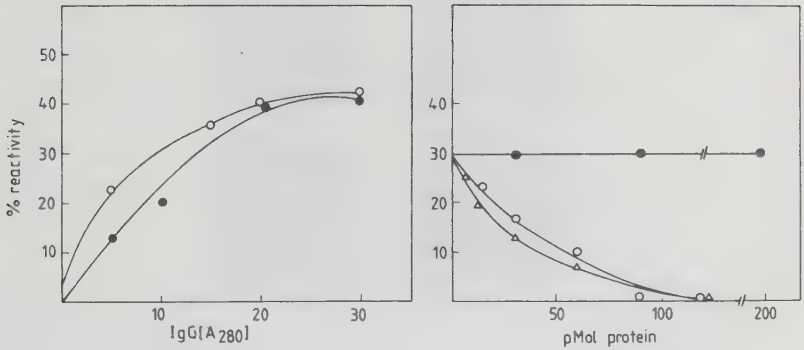


Fig.38. Sucrose gradient centrifugation with anti-L5. (a) Reactivity of 50S subunits with anti-L5 preparations obtained from R1081 (o—o) and H142 (●—●). (b) Inhibition of ribosome-antibody complex formation. 1.0 A_{280} of IgG from R1081 were preincubated with increasing amounts of single protein L5 (Δ—Δ), TP70 (o—o), TP50 lacking protein L5 (●—●).

5.7.3. Localization of protein L5 by immunoelectron microscopy

Fig.40a shows a field of 50S subunits which had been reacted with anti-L5. Most of the subunits (90%) are present as dimeric immunocomplexes. Monomeric immunocomplexes were rare, accounting for only 4% of the subunits. Occasionally, pairs of subunits joined by two IgG molecules are observed. Typical immunocomplexes are marked by arrows.

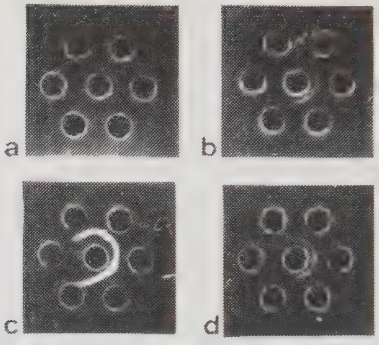


Fig.39. Double immunodiffusion. The peripheral wells contained 10-20-40-60 µg of TP50 lacking L5 (a and b) or TP50 (c and d); The centre wells contained antiserum specific for protein L5 (a and c) or, as a control, protein L3 (b and d).

Fig.41. The location of protein L5 on the three-dimensional model of the 50S subunit.

In the dimers, the subunits are seen both in the crown and in the kidney projection, the kidney forms being less frequent than in preparations of isolated 50S subunits. In the crown projection, the antibody binds to the central protuberance (Fig.40b,c). Close inspection of the immunocomplexes reveals that the Fab arm of the connecting molecule is only partly visible. Dimers like those shown in Fig.40c are representative of 20% of the immunocomplexes, in which two crown forms are connected by an antibody. The two subunits are very close and in most cases the antibody molecule is not fully visible. This observation indicates that the antibody attachment point is not at the top of the central protuberance, but rather at its base. In these dimers the antibody binding site is between the central and the L1 protuberances.

In the kidney view, the antibody binds close to the pointed end on the side of the subunit which in the 70S ribosome faces the 30S particle (Fig.40d). Crown views which are connected with kidney views are shown in row e of Fig.40. Aberrant antibody binding sites were not observed.

These data allow the three-dimensional localization of protein L5 at the lower part of the central protuberance on the side which faces the L1 protuberance (Fig.41)

The two anti-L5 preparations were tested for their reactivity with 70S ribosomes and in both cases no 70S-IgG-70S complexes were formed. This result is in good accordance with the localization of protein L5 on the interface side of the large subunit, as shown in Fig.41.

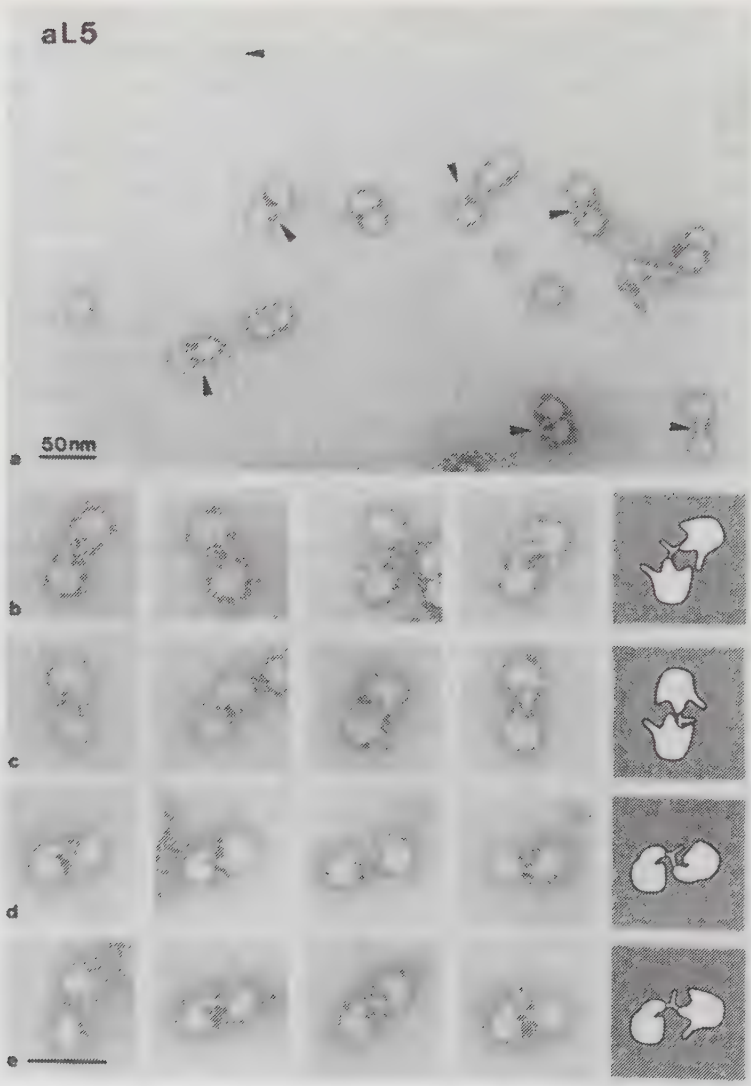


Fig.40. Electron micrographs of 50S subunits reacted with anti-L5. (a) General view with typical immunocomplexes marked by arrows, (b-e) individual immunocomplexes in which the subunits are seen in the crown form (b,c) and in the kidney form (d). (e)Dimers in which a crown form is linked with a kidney form.

5.8. Protein L27

5.8.1. Reactivity of 50S subunits with anti-L27

For the localization of protein L27 two antisera raised in rabbit were available which formed stable immunocomplexes with 50S subunits (Table 12). Anti-L27 R944 was used for the subsequent experiments. The reactivity of 50S subunits with this IgG is shown in Fig.42a.

5.8.2. Antibody specificity for protein L27

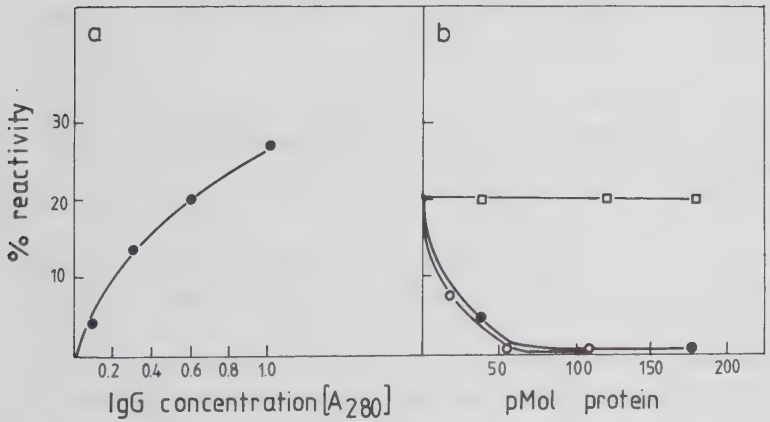


Fig.42. Sucrose gradient centrifugation with anti-L27. (a) Reactivity of 50S subunits with anti-L27 R944 plotted against antibody concentration. (b) Inhibition of dimer formation by preabsorbing 0.6 A_{280} IgG with increasing amounts of purified protein L27 (o–o), TP70 (●–●), TP70 from mutant ES 5 which lacks protein L27 (□–□).

Preliminary evidence for the specificity of the IgG was obtained by incubation with 50S core particles which lack protein L27 (Table 8): no immunocomplex formation was detected in the sucrose gradient (Table 10). This experiment, although not sufficient to demonstrate the specificity of the antibody, excluded the presence of contaminating antibodies directed against proteins present in the core particles. The formation of immunocomplexes is completely abolished by preincubation of anti-L27 with 0.5 µg of single protein L27, corresponding to 55 pmol protein (Fig.42b). A mutant ES 5 whose ribosomes lack protein L27 has been characterized. Such ribosomes are not able to form complexes with anti-L27 (data not shown). Accordingly, preincubation on anti-L27 with increasing amounts of TP70 extracted from ribosomes of the mutant, does not affect dimer formation (Fig.42b). In contrast, preabsorption with 150 µg TP70 from wild type completely abolishes the formation of immunocomplexes (Fig.42b). This latter set of experiments rules out cross-reaction of the antibody wih ribosomal proteins other than L27.

5.8.3. Localization of protein L27 by immunoelectron microscopy

A general field of 50S subunits reacted with antibodies against protein L27 is shown in Fig.43a. From the evaluated 50S subunits, 77% formed dimeric and 4% formed monomeric immunocomplexes, while 19% had no antibody attached. Some typical antibody-ribosome complexes are indicated by arrows.

The analysis of individual images at higher magnification reveals that in most dimers (75%) the two subunits lie very close to one another; in these immunocomplexes the IgG molecule is not visible at all, making it impossible to determine the antibody binding site (Fig.43b). By screening a large number of micrographs, a sufficient number of dimers could be de-

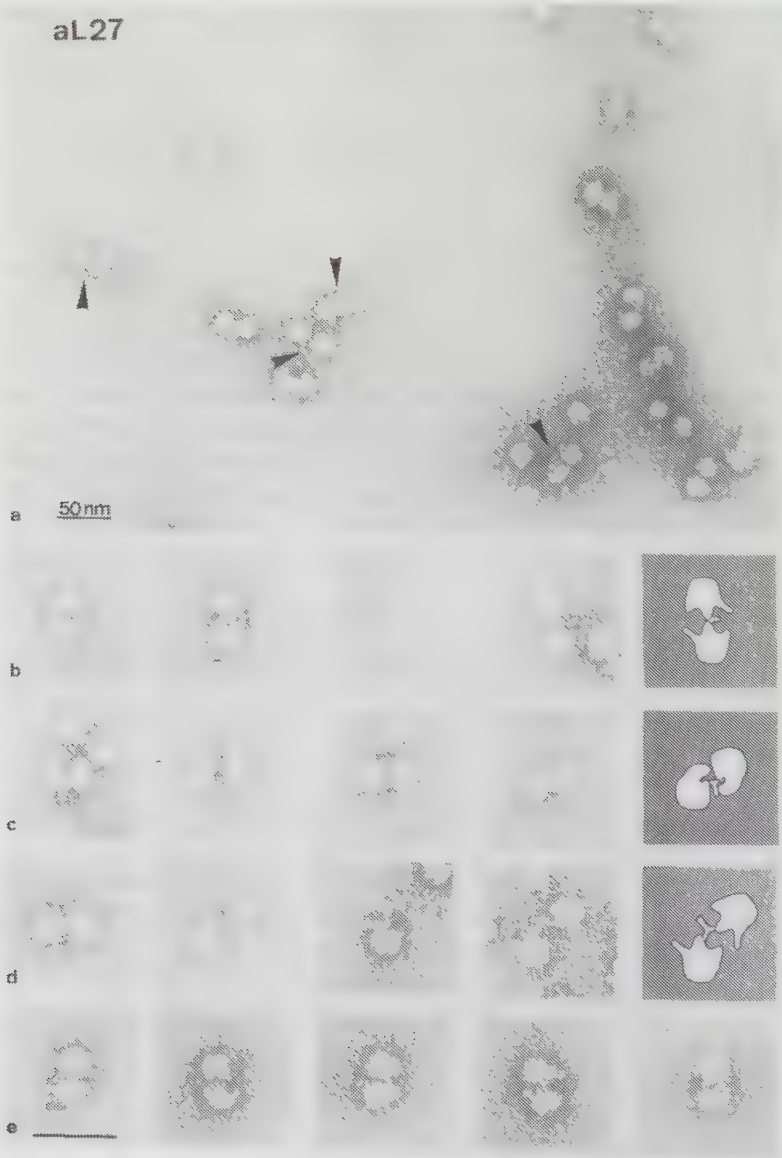
tected in which the connecting antibody was visible; these immunocomplexes were used to locate the antibody attachment site. Pairs of subunits in which the particles are in the crown form have the antibody attached to the central protuberance (Fig.43d). Since the IgG is only partly visible, its binding site must be positioned away from the subunit contour lines, likely at the base of the central protuberance.

In the kidney projection, the antibody binds to the interface side, close to the notch (Fig.43c).

In several dimers, the subunits are seen in projections which are usually not observed in standard preparations of 50S subunits (Fig.43e). We interpret these complexes as the subunits facing each other with their flat sides; the antibody molecule is not visible.

Two IgG molecules connecting two subunits simultaneously have not been observed; however, it cannot be excluded that those immunocomplexes in which the two subunits are very close to each other have two IgG molecules bound.

Fig.43. Electron micrographs of 50S subunits reacted with anti-L27. (a) Overall field, (b-e) selected micrographs showing the subunits in the crown form (b and d), in the kidney form (c) and in rare projectional forms (e).


aL27
a
50 nm
b
c
d
e

The interpretation of the electron micrographs obtained with anti-L27 was particularly difficult. Images which help in the correct positioning of the antibody attachment point are shown in Fig.44 together with interpretative schemes. Dimers as shown in Fig.44a are characteristic for 50S-anti-L27 complexes: the two crown forms are very close and the L1 protuberance of one crown form is in contact with the central protuberance of the other. The scheme shows our interpretation of such dimers, namely that the two subunits are connected by an antibody which binds at the base of the central protuberance and is therefore completely obscured by the subunits. The same antibody binding site can be deduced from the dimer shown in Fig.44b in which one of the Fab arms is visible in its full length. The two electron micrographs shown in Fig.44c,d are characteristic for immunocomplexes in which a kidney view is connected by the IgG molecule with a crown view. The two subunits are in close contact to each other, and in the kidney form the antibody molecule binds in the region of the notch.

5.8.4. Three-dimensional localization of protein L27

The localization of L27 on the surface of the 50S subunits as deduced from the observations reported in the preceding section is given in Fig.45. On the 3-D model the protein is located in the middle of the interface side in a region extending from the lower part of the central protuberance towards the base of the particle. The width of the area dashed on the model reflects some uncertainty in defining the boundary of the antibody binding site.

According to this localization, anti-L27 does not form immunocomplexes with 70S ribosomes.

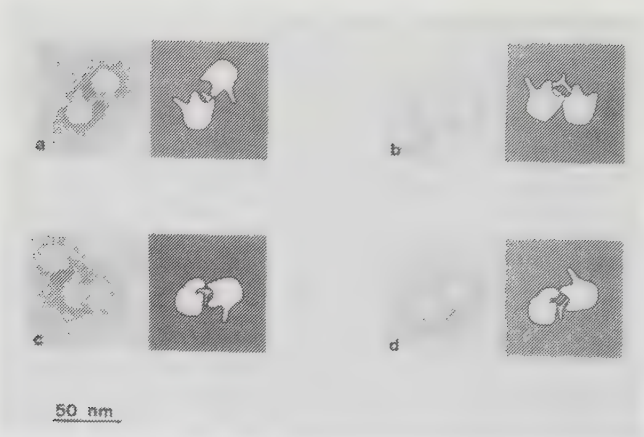


Fig.44. Selected electron micrographs of individual 50S anti-L27 complexes. For details see text.

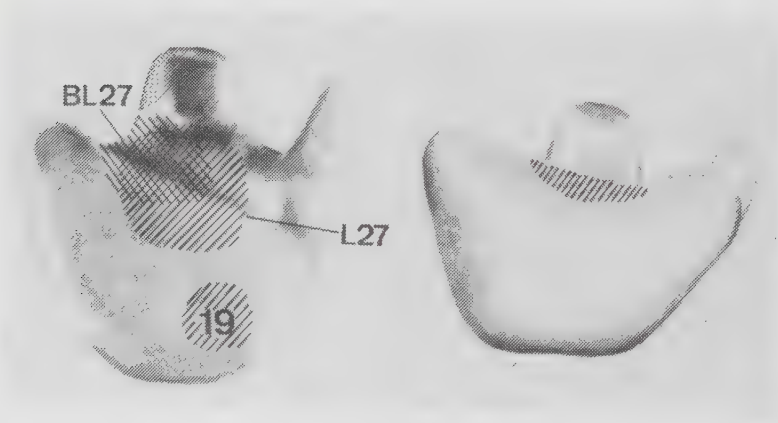


Fig.45. The location of protein L27 on the three-dimensional model of the 50S subunit. The locations of proteins BL27 from Bacillus subtilis and L19 are also indicated. The dotted area represents the location of L27 as proposed by Lake and Strycharz (1981).

5.8.5. Study on the relative locations of proteins L27 and L19 on the 50S subunit

The mapping of L27 in the middle part of the interface side of the large subunit positions this protein in an area of the 50S particle where only a few proteins have been localized to date. Recently, epitopes of protein L19 have been localized on the base of the subunit below the L7/L12 stalk (Stöffler et al., 1984). Anti-L19 antibodies are not reactive with 70S ribosomes, consistent with a location of L19 at the interface side of the subunit.

Since it was very difficult to define the lower boundary of the binding site of anti-L27, we hoped to refine our localization by gaining more knowledge about the relative location of L19 and L27. For this purpose, we studied the effect of incubating a mixture of antibodies specific for protein L27 and L19 with 50S subunits. Immunocomplexes obtained with anti-L27 sediment faster than those obtained with anti-L19 (compare Fig.46b and c). When 50S subunits are incubated with anti-L27 and anti-L19 simultaneously, the dimer peak containing the 50S-anti-L27 immunocomplexes has the same height as in the control, while the dimer peak containing 50S-anti-L19 immunocomplexes is strongly reduced, compared with the control (Fig.46). Electron micrographs of samples from the two dimer peaks show that the "L19" peak contains exclusively antibody binding to the region where protein L19 has been localized and the "L27" peak contains mainly dimers joined by IgGs binding to the L27 site (results not shown). If the antibody mixture (anti-L27 + anti-L19) was preincubated with increasing amounts of purified protein L27, the "L27 peak" vanished while the height of the "L19 peak" increased. This result suggests that the two antibodies compete for binding to the 50S subunit. Thus, it seems likely that proteins L27 and L19 are close neighbours in the three-dimensional structure of the large ribosomal subunit.

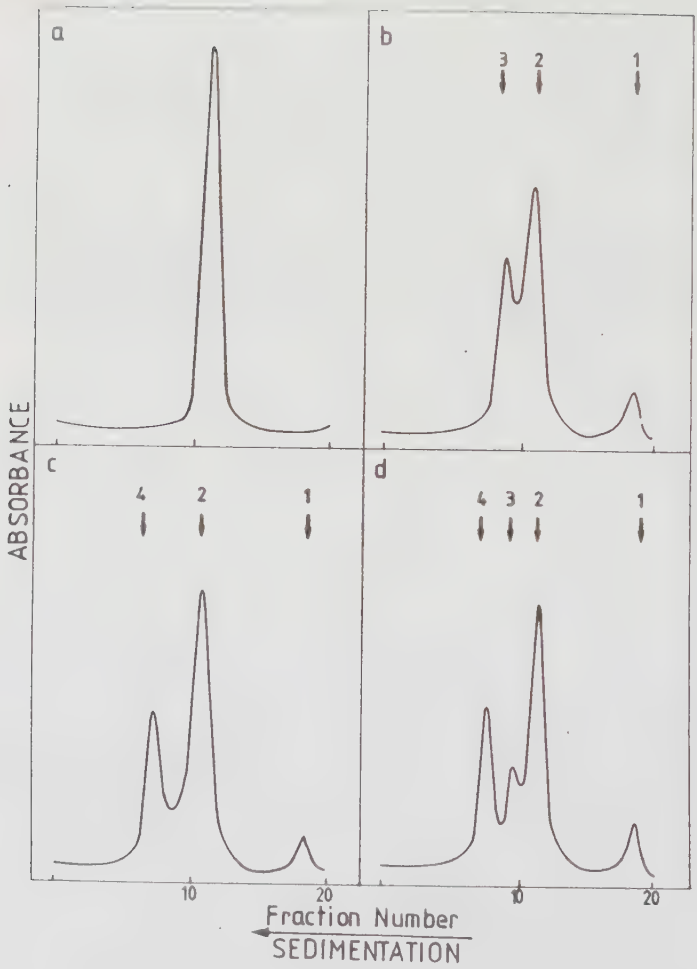


Fig.46. Reactivity of 50S subunits (a) with: 2.0 A_{280} anti-L19 (b), 0.6 A_{280} anti-L27 (c), a mixture of the two IgGs (d). Peak 1: IgG, peak 2: 50S subunits, peak 3: anti-L19 linked dimers, peak 4: anti-L27 linked dimers.

5.8.6. Reconstitution of mutant ES 5 of E.coli with protein BL27 from Bacillus subtilis

When this study was started, the IgG specific for protein L27 was not yet available. Therefore, we tried to localize protein BL27 on the surface of 50S subunits of Bacillus subtilis by the corresponding antibody. However, electron micrographs of the immunocomplexes obtained showed antibody binding in different regions of the 50S subunit (Fig.47). This observation indicated the presence of contaminating antibodies in the anti-BL27 preparation. In this case, it was not possible to purify the antibody by affinity chromatography since protein BL27 was not available in sufficient amounts. To overcome the problems arising from the presence of contaminating antibodies, we decided to reconstitute 50S subunits of the mutant ES 5 from E. coli which lacks protein L27, with protein BL27 and to localize BL27 on the surface of the reconstituted subunits.

When 3.0 A_{280} units of anti-BL27 are reacted with 50S subunits of Bacillus subtilis, 15% of the subunits dimerize (Fig.48). The antibody also reacts with 50S subunits from mutant ES 5 reconstituted with purified protein BL27, although the reaction is somewhat reduced (Fig.48). In contrast, no dimer formation is observed when the antibody is reacted with 50S subunits from E. coli wild type.

This experiment clearly establishes that the dimerization of the reconstituted subunits is exclusively dependent on the presence of protein BL27 in these subunits and excludes the occurrence of cross-reactions with any of the other 50S subunit proteins of E. coli.

In the electron micrographs, the shape of the reconstituted particles is identical to that of 50S subunits from E. coli (compare Fig.49 with Fig.1).

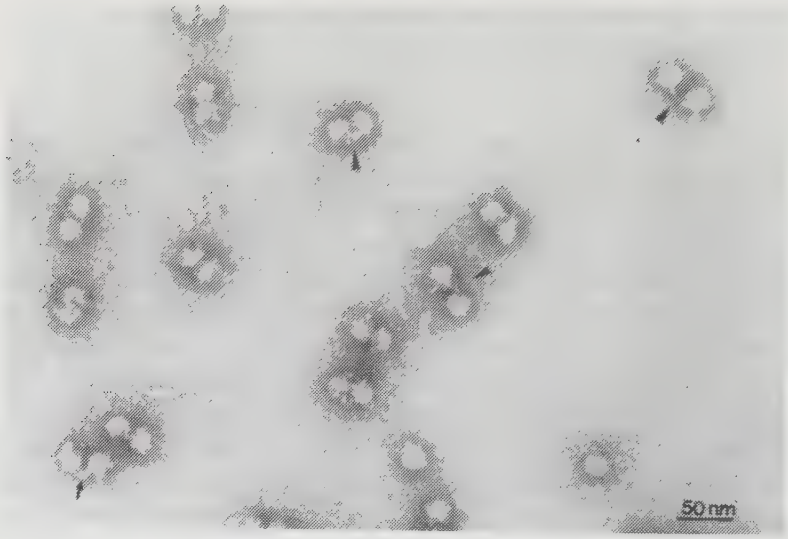


Fig.47. Electron micrographs of 50S subunits of Bacillus subtilis reacted with anti-BL27.

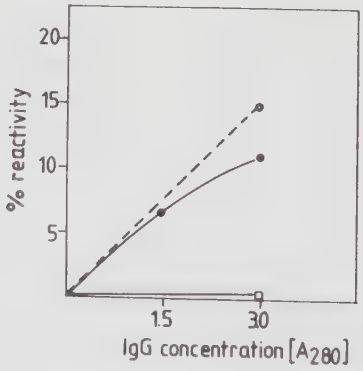


Fig.48. Reactivity of anti-BL27 with: 50S subunits from Bacillus subtilis (o–o), 50S subunits from mutant ES 5 (□–□) and 50S subunits from mutant ES 5 reconstituted with BL27 (●–●).

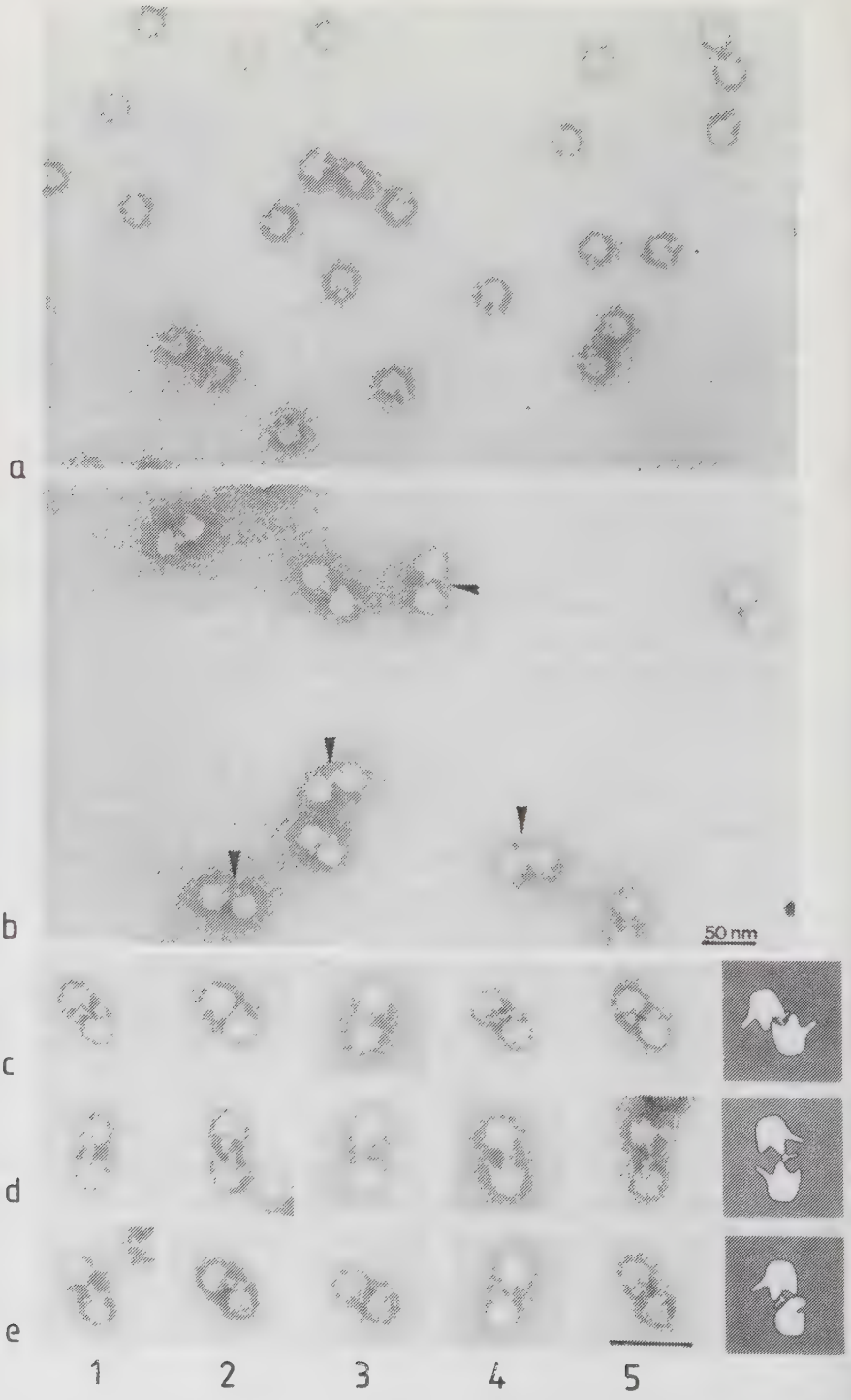
Reconstituted particles reacted with antibody against protein BL27 are shown in Fig.49b-e. 90% of the subunits are present as dimeric immunocomplexes, while 50S-IgG monomers are not observed. Pairs of subunits linked by two IgG molecules are not detected.

Selected micrographs are shown in Fig.49c-e. When the subunits are seen in the crown view, two kinds of dimers are observed with approximately the same frequency. In the first type, the antibody molecule is not visible and the subunits are very close with the central protuberances facing each other (Fig.49c). Our interpretation is that these pairs are connected by an antibody attaching at a site far away from the subunit outlines as suggested in the scheme. In the other type of immunocomplexes, the two subunits are joined by the antibody via their central protuberances. Since the Fab arm of the IgG molecule is only partly visible, the antibody binding point is likely to be at the base of the central protuberance (Fig.49d).

In the kidney view, the antibody binds to the convex side of the particle, close to the pointed end (Fig.49e). In contrast, in the 50S-anti-L27 complexes, the antibody binding site was on the flat side of the subunit (5.8.3.).

The epitope of protein BL27 in the reconstituted *E. coli* mutant is thus at the lower part of the central protuberance. Its localization on the three-dimensional model of the 50S subunit is given in Fig.45.

Fig.49. (a) Electron micrographs of 50S subunits from mutant ES5 reconstituted with protein BL27. (b-e) Reconstituted subunits after reaction with anti-BL27.


a
b
50 nm
c
d
e
1
2
3
4
5

6. DISCUSSION: TOPOGRAPHY OF THE 50S SUBUNIT

6.1. Core particles from 50S subunits

When I began my work on the structure of the 50S subunit, many of the 50S proteins had not yet been localized by immunoelectron microscopy since the available IgGs did not form immunocomplexes with the subunit. At the time, the most probable explanation for this was that the antibodies used might be directed against epitopes which were not exposed on the surface of the subunit. Therefore protein depleted core particles were prepared and characterized.

When 50S subunits were treated with 1.5 M LiCl, core particles were obtained which were depleted of 14 proteins, correponding to approximately one-half of the proteins of the 50S subunit. In contrast to the results reported for the 30S cores (4.1.), subparticles prepared by different authors with the same LiCl concentration are very similar in their protein content (Table 13).

The proteins which are removed by LiCl treatment are late assembly proteins, most of which belong to the so-called "L15 domain" consisting of several proteins which are considered to depend on L15 for their assembly (Nierhaus, 1980). This group of proteins has also been found to be particularly exposed to chemical and enzymatic modification (Crichton and Wittmann, 1971; Moore, 1971; Kahan and Kaltschmidt, 1972; Litman and Cantor, 1974). These observations are consistent with a location of these proteins near the surface of the subunit.

Table 13: Protein composition of core particles from 50S subunits

Protein	1.5 M LiCl [a]	1.0 M LiCl [b]	2.0 LiCl [b]	1.5 M LiCl [c]
L1	-	red	-	-
L2	+	+	red	-
L3	+	+	+	+
L4	+	+	+	+
L5	+	+	+	red
L6	-	-	-	-
L7/L12	-	red	-	-
L9	-	+	-	-
L10	-	-	-	-
L11	-	-	-	-
L13	+	+	+	+
L14	+	+	red	-
L15	-	+	-	-
L16	-	-	-	-
L17	+	+	+	+
L18	+	+	+	-
L19	+	+	+	+
L20	n.d.	+	+	-
L21	+	+	+	+
L22	+	+	+	+
L23	+	+	+	+
L24	+	+	+	+
L25	-	+	-	-
L27	-	red	-	-
L28	-	-	-	-
L29	+	+	+	+
L30	-	+	+	-
L31	n.d.	n.d.	n.d.	-
L32	+	+	+	red
L33	-	+	+	-
L34	+	n.d.	n.d.	n.d.

red = protein detected in reduced amount; n.d. = not determinde; (a) this work; (b) Boublik et al., 1978; (c) Homann and Nierhaus, 1971.

The shape of the core particles as observed in the electron microscope reflected the topography of the removed proteins, the protein depletion mainly affecting the two side protuberances from which a considerable number of proteins was depleted. Thus, proteins L1 and L9 were removed from the L1 protuberance and proteins L7/L12, L6, L10 and L11 from the region of the stalk. For this reason, most of the particles were round-shaped and lacked clearly recognizable structures. Evidence of unfolding or degradation of the particles was not found. In general, the 50S core particles seem to maintain a higher compactness than the 30S subparticles (4.2.).

Our own observations were compared with those reported by Boublik in a study on the structure of core particles obtained by incubation of 50S subunits with 0.5 M to 6.0 M LiCl (Boublik et al., 1978). Since the core particles prepared for this work are very similar in their protein content to those obtained with 2.0 M LiCl by Boublik (Table 13), a direct comparison between the two preparations was possible. Boublik described the 2.0 M LiCl cores as reduced in their size and mainly formed by a compact core from which protrudes an attenuated central protuberance; the side protuberances were completely missing. Our core particles mostly showed a better conserved structure and were rather similar to the core particles which were prepared by Boublik with 1.0 M LiCl, and which lacked five proteins completely and contained another three proteins in reduced amounts (Table 13).

As in the case of the 30S core particles (4.2.), there is some disagreement about the structure of the core particles and, as a consequence, about the role of the 50S proteins in determining the structure of the subunit. Boublik reported that after exposure to 4.0 M or higher concentrations of LiCl, the majority of the particles were unfolded and did not show any similarities with the 50S subunit. In contrast, it was shown that isolated molecules of 23S RNA in the presence of polyamines

are able to self-pack in a structure bearing morphological similarity with the 50S subunit (Vasiliev and Zalite, 1980). We have not attempted to investigate the effect of a more extensive protein depletion on the structure of the core particles. However, based on our electron microscopic observations and on the results obtained with 30S core particles (4.2.), we believe that the 50S subparticles should also be able to maintain the characteristic folding of the 50S subunit.

By protein depletion, the 50S subunits were converted into symmetric structures lacking distinctive morphological landmarks. For this reason, a further characterization of their structural organization based on the localization of proteins as done in the case of the 30S core particles (3.5.) was not performed. The localization of proteins on the surface of these core particles would be possible either by double antibody labelling or after partial reconstitution of some structural landmarks, e.g., the L7/L12 stalk, providing the points of orientation which are necessary in order to determine the antibody binding site.

6.2. Accessibility of ribosomal proteins on the surface of 50S subunits and of core particles

By various techniques such as enzymatic and chemical modification (Crichton and Wittmann, 1971; Moore, 1971; Kahan and Kaltschmidt, 1972; Litman and Cantor, 1974) as well as by antibody binding studies (Morrison et al., 1977), it was shown that most of the proteins of the 50S subunit are exposed at the surface of the particle.

However, a large number of antibody preparations specific for single ribosomal proteins of the 50S subunit did not form immunocomplexes when reacted with 50S subunits. These antibodies

were incubated with core particles in order to test if they were directed against antigenic determinants which are masked by other proteins. This hypothesis was suggested also by the observation that none of the proteins that bind to the 5'-end of the 23S RNA (L4, L13, L20, L22 and L24) had been mapped by antibody label. This group of proteins is involved in the early assembly of the subunit (Nierhaus, 1980). Thus, it was conceivable that they form a "core" inside the subunit and are therefore not exposed at its surface. Out of a number of antibodies which did not dimerize 50S subunits, only two were reactive with core particles (5.1.4.). It was not possible to demonstrate the specificity of these two antibodies by the standard controls; however, this result showed that the IgGs in question were specific for antigenic determinants which become accessible for antibody binding only after depletion of part of the proteins. Most of the antibodies tested did not dimerize either intact subunits or core particles (5.1.4.). Thus, as already discussed in more detail for the 30S subunit (4.3.), it is likely that the lack of reactivity with both intact particles and cores depends on the characteristics of the antibody rather than on the inaccessibility of the reactive epitopes.

6.3. Localization of ribosomal proteins at the surface of the 50S subunit

Altogether, six proteins of the 50S subunit, namely L4, L5, L6, L9, L15 and L27 were localized by immunoelectron microscopy. Proteins L5, L15 and L27 were localized at the interface region of the subunit, in a domain extending from the lower part of the central protuberance to the middle of the subunit; protein L9 was mapped on the base of the L1 protuberance, protein L6 at the region from which the stalk originates and protein L4 on the back of the large subunit. The locations of these proteins on the three-dimensional model of the 50S subunit are summarized in Fig.50.

Fig.50. The locations of proteins L4, L5, L6, L9, L15, and L27 on the three-dimensional model of the 50S subunit.

The specificity of all the antibodies used has been assessed by the control experiments described in 5.2.. For the first control, the formation of immunocomplexes must be abolished by preabsorption of the antibody with the isolated antigen protein. It was observed that in the experiments performed for the various IgGs, different amounts of purified individual proteins were required in order to saturate the antibody. Thus, the formation of immunocomplexes with anti-L27 and anti-L15 was inhibited by purified protein in stoichiometric ratio with the amount of protein present on the 50S subunits. In contrast, purified protein L6 was necessary in large molar excess in order to saturate the anti-L6 IgG. This variability in the amount of protein needed for the complete absorption of the antibody can be due to various reasons. The most likely of them is that part of the antigenic determinants present on a protein might be destroyed during the isolation procedure. In the case of antibodies which are specific for protein conformation, the saturation is only achieved by proteins maintaining their native conformation. For example, anti-L6 was saturated by native protein L6 but not by other L6 preparations

extracted under denaturating conditions (5.4.2.). Crossreactions of the IgG with ribosomal proteins other than the protein in study were excluded by preabsorption of the antibody with total protein mixtures which lacked the protein in question. A simple procedure to prepare such mixtures from total protein by immunoaffinity chromatography has been set up to demonstrate the specificity of anti-L4 and anti-L5 for the respective proteins. An important advantage of having a quick procedure to prepare protein mixtures in which a given protein is missing is demonstrated by the experiments carried out with anti-L4. The absorption of this IgG with protein L4 had shown that the antibody preparation contained also contaminating antibodies specific for proteins other than L4; however, preabsorption of the IgG with TP50 lacking protein L4 allowed us to eliminate the contaminating antibodies and to select the only anti-L4 specific antibodies before reaction with the 50S subunits.

6.4. Organization of the ribosomal proteins in the 50S subunit

Altogether 17 proteins have now been located at distinct sites on the surface of the 50S subunit of the *E. coli* ribosome (Fig.51a). The ribosomal proteins appear to be clustered in domains on the surface of the subunit. The central protuberance contains antigenic determinants of proteins L5, L18 and L25, each binding to the 5S RNA. These data are in agreement with the placing of the 3'-end of the 5S RNA at the central protuberance (Shatsky et al., 1980; Stöffler-Meilicke et al., 1981). Epitopes of proteins L15 and L27 are on the base of the protuberance. A second domain is on the broad lateral protuberance and comprises proteins L1 and L9. The stalk contains antigenic determinants of the proteins L7/L12, whereas the protuberance from which the stalk originates contains proteins L6, L10 and L11. A determinant of protein L19 has been found slightly separated from this domain. A cluster of proteins including L4, L23 and L29 has been localized

on the back side of the subunit. Up to date, L17 is the lone protein which has been mapped on the convex side of the subunit facing the stalk.

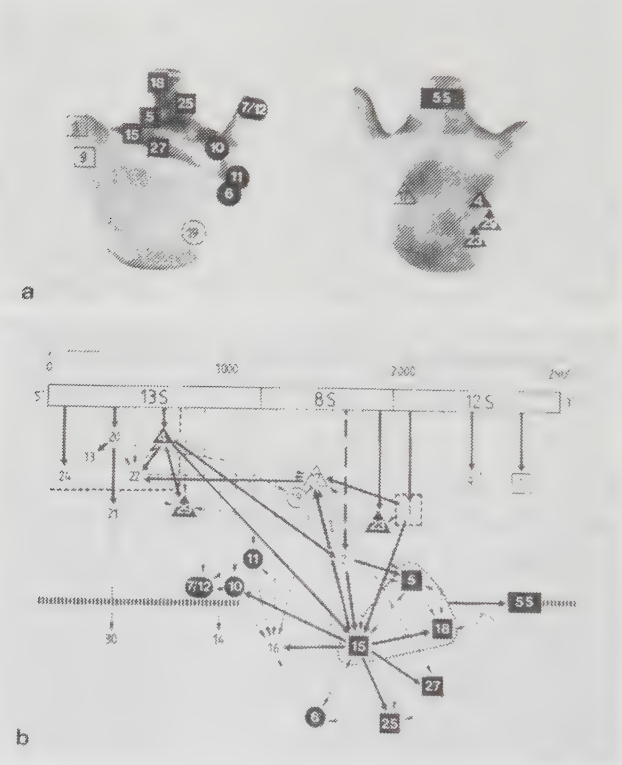


Fig. 51. (a) Location of ribosomal proteins of the 50S subunit as determined by immunoelectron microscopy (Stöffler and Stöffler-Meilicke, 1984; this work). The 3'-end of the 5S RNA has also been mapped by immunoelectron microscopy (Shatsky et al., 1980; Stöffler-Meilicke et al., 1981). (b) Assembly map of the 50S subunit (Röhl and Nierhaus, 1982). Proteins which have been localized in the same domain on the 50S subunit are indicated by the same symbols in both (a) and (b).

The distribution of the proteins in domains agrees well with the results of other studies on protein topography, e.g. protein-protein cross-linking (Liljas, 1982; Nierhaus, 1982; Wittmann, 1983).

Good agreement is also found between the locations of the proteins reported above and the structure of the 50S core particles as observed in the electron micrographs (5.1.3.). The subparticles have rounded contours according to the topography of the deleted proteins: L1 and L9 are removed from the L1 protuberance; L6, L10, L11 and L7/L12 from the region of the stalk. In addition, proteins L15 and L27 are deleted from the basis of the central protuberance; particles with this protuberance reduced in size have also been observed.

When the topographical data are compared with the assembly map of the 50S subunit (Röhl and Nierhaus, 1982), a striking coincidence is observed between the location of the proteins in the subunit and their assembly dependence. The proteins which belong to the same structural domain in the subunit are also correlated in the assembly map (Fig.51). Thus, for example, proteins L1 and L9 which bind to the same region of the 23S RNA, are located in a common domain. Similarly, the group of proteins surrounding L15 in the assembly map have been localized at the central protuberance or at its base. These observations strongly indicate that functional relationships reflect structural neigbourhoods in the subunit.

6.5. Comparison of the localization of proteins L4, L5, L6, L9, L15, and L27 with the results of other methods

In the following sections, our localization of the six proteins by immunoelectron microscopy shall be compared with all the data available about these proteins, as well as with the

Table 14. Topographical data on the location of proteins L4, L5, L6, L9, L15 and L27

Protein	Method	Result	Reference
L9	Cross-linking	L9-L2	Traut et al., 1980
		L9-L28	
		L9-L7/L12	Traut et al., 1983
	RNA-protein fragments	L9-L1	Roth and Nierhaus, 1975
L6	Cross-linking	L6-L2	Traut et al., 1980
		L6-L7/L12	
		L6-L11	
		L6-L17	
	Protein complexes	L6-L5-L11	Wystup et al., 1979
L4	Cross-linking	L4-L11	Traut et al., 1980
		L4-L14	
	RNA-protein fragments	L4-L3-L24	Newton and Brimacombe, 1974
	Neutron scattering (distances)	L4-L2 : 115 A°	Nierhaus et al., 1983
		L4-L16: 80 A°	
		L4-L23: 45 A°	Nowotny, personal comm.
L15	Protein complexes	L15-L2	Wystup et al., 1979
		L15-L27	
		L15-L17-L27	

Continuation: Table 14

Protein	Method	Result	Reference
L5	Cross-linking	L5-L2	Traut et al., 1980
		L5-L3	
		L5-L7/L12	
		L5-L11	
		L5-L17	
		L5-L23	
		L5-L25	
		L5-L18	Fanning and Traut, 1981
		L5-L31	
		L5-S9	Cover et al., 1981
		L5-S13	
		L5-S19	
	Protein complexes	L5-L6-L11	Wystup et al., 1979
	RNA-protein fragments	L5-L18	Roth and Nierhaus, 1975
		L5-L18-L25	Newton and Brimacombe, 1974
L27	Cross-linking	L27-L16	Traut et al., 1980
		L27-L17	
		L27-L29	
		L27-S9	
	Protein complexes	L27-L15	Wystup et al., 1979
		L27-L17-L15	
		L27-L16	

results of other immunoelectron microscopic studies and with the topographical data provided by other experimental approaches.

Various techniques have been employed for the analysis of the quaternary structure of the ribosome. The techniques which are most widely used in studying the arrangement of the proteins within the ribosome are, besides immunoelectron microscopy, neutron scattering, protein-protein cross-linking with bifunctional reagents, fragmentation of the ribosome with RNase, isolation of protein complexes, and singlet-singlet energy transfer between fluorescent dyes coupled to two different proteins. Characteristics and results of these methods have been reviewed by Wittmann (1983). The comparison of the results obtained by different methods delivers complementary information about the arrangement of the proteins within the ribosome. Thus, for example, immunoelectron microscopy yields information on the location of ribosomal components at the surface of the particles, whereas neutron scattering depicts the internal architecture of the ribosome.

All the data about the topography of proteins L4, L5, L6, L9, L15 and L27 as obtained by various topographical methods are summarized in Table 14 and shall be discussed in more detail in the sections relevant to each protein.

6.5.1. Protein L9

The location of L9 on the wider lateral protuberance, below protein L1, is in good accordance with the finding that both proteins bind to the 3'-end of the 23S RNA (Chen-Schmeisser and Garrett, 1976). Moreover, a ribonucleoprotein fragment containing both proteins L1 and L9 has been isolated by treatment with RNase; proteins found together in such fragments are

considered to be neighbours in the intact particle. Only a few proteins, namely L2, L7/L12 and L28 have been cross-linked with L9 (Table 14). Out of these proteins, L2 and L28 have not yet been localized. The cross-link L9-L7/L12 occurred only rarely (Traut et al., 1983) and is hardly compatible with the location of the two proteins as determined by immunoelectron microsocopy (Fig.51a).

6.5.2. Protein L4

We localized protein L4 in a relatively large region of the 50S subunit, according to the multiple antibody binding sites observed far apart from each other in the electron micrographs. Accordingly, gel filtration experiments showed that L4 has a moderately elongated shape (Wills and Dijk, personal communication). Similar results were also obtained by neutron scattering: a radius of gyration of about 20 A° corresponding to an axial ratio of 3:1 was found for protein L4 (Nierhaus et al., 1983).

Preliminary results obtained in our group, indicated that protein L4 was at the back of the subunit below L1, at the same site as described in this work (Noah et al., 1982). However, the specificity of the antibody used could not be demonstrated at the time.

Only 15% of the 50S subunits were reactive with the antibody we used for the localization of protein L4 (5.5.1.). Apparently, the antibody can only react with a fraction of the 50S population, and this fraction could possibly consist of functionally inactive subunits. However, we demonstrated that the arrangement of the ribosomal proteins is the same in both active and inactive subunits (4.2.); therefore, the degree of activity of the particles should not be crucial for the localization

of L4. Protein L4 is one of the few proteins which remain bound to ribonucleoprotein core particles derived from 50S subunits by treatment with 4.0 M LiCl (Homann and Nierhaus, 1971). It binds directly to the 5'-end of the 23S RNA and constitutes, together with the other early assembly proteins, the "core" of the 50S subunit and it supports the assembly of many other proteins (Röhl and Nierhaus, 1982). This central position of L4 within the subunit could be responsible for the low reactivity of anti-L4 antibodies.

By neutron scattering experiments, a distance of 45 A° was found for the pair L4-L23 (Nowotny, personal communication) which fits very well the location of the two proteins by antibody label (Fig.51a). For the pairs L4-L2 and L4-L16 distances of 120 A° and 70 A° were observed (Nierhaus et al., 1983); up to date, neither L2 nor L16 have been localized.

L4 has been suggested to be involved in peptidyltransferase activity (Nierhaus, 1982; Liljas, 1982). Our localization of L4 on the back side of the subunit in the cluster also comprising L23 and L29, situates this protein relatively far from the peptidyltransferase center which has been localized on the angle between the central and the L1 protuberance (Lührmann et al., 1981; McKuskie-Olson et al., 1982).

6.5.3. Protein L6

According to its localization as described in 5.4.3., L6 contributes to a domain which is situated in the region from which the L7/L12 stalk originates. This domain comprises also proteins L10 and L11 (Fig.51a). All these proteins are directly or indirectly involved in the binding of the protein synthesis factors, such as initiation factors (IF)-2, elongation factors (EF)-G and EF-tu, release factors (RF)-1 and

RF-2 (Girshovich et al., 1981; Stöffler et al., 1982; Liljas, 1982).

Our localization is in agreement with that obtained by the use of hapten specific antibodies (Stöffler-Meilicke et al., 1983a). In this study, the lone cysteine residue in position 86 has been modified with 5-iodoacetamidofluorescein and the hapten has been localized by fluorescein specific antibodies. This approach showed that the cysteine 86 of protein L6 is at or close to the surface of the ribosomal subunit. More precise data on the arrangement of protein L6 in the subunit could possibly be obtained using fragment specific antibodies.

L6 has been crosslinked with proteins L2, L7/L12 and L17 (Table 14). Our localization fits well the cross-linking with L11 and L7/L12 but not with L17 (Fig.51a). Protein L2 has not yet been mapped.

6.5.4. Protein L15

L15 has been located at the angle between the L1 and the central protuberance.

Only few data have been reported describing the topographical relationships of L15 with other ribosomal components (Table 14). The isolation of RNA-protein complexes with protein L27 fits well the location of both proteins as proposed in this work (Fig. 51a). Protein L15 has not so far been cross-linked to any other large subunit protein (Table 14). This is surprising, in view of the pivotal role that has been assigned to this protein in the *in vitro* assembly of the 50S subunit (Nierhaus, 1982).

L15 has, together with other proteins, been described as being important for peptidyltransferase activity (Nierhaus, 1982; Liljas, 1982). Our localization would agree with the topographical localization of the peptidyltransferase center (Lührmann et al., 1981; McKuskie-Olson et al., 1982). However, questions about the function of protein L15 arise from the viability of a mutant lacking this protein (Lotti et al., 1983). The mutant showed normal subunit profiles and had all other ribosomal proteins in normal amounts. The viability of this mutant lacking L15 excludes the possibility that this protein is essential in the catalysis of the peptidyl transfer. However, since the rate of poly(U)-dependent polyphenylalanine synthesis of the mutant is significantly lower than that of the wild type (Tate W., Hasenbank R. and Stöffler G., unpublished results), it is likely that protein L15 may be involved in facilitating the proper positioning of the tRNAs bound to the A and the P sites.

6.5.5. Protein L5

We localized L5 at the lower part of the central protuberance on the side which faces the 30S subunit in the 70S ribosome.

A large amount of information about the topography of L5 within the ribosome has been obtained by different experimental approaches (Table 14).

L5 has been crosslinked with many proteins of the 50S subunit, among them proteins L18 and L25. The proximity of L5, L18 and L25 is also supported by the isolation of RNA-protein fragments containing these proteins (Table 14). Moreover, all three proteins bind to the 5S RNA (Erdmann, 1976; Garret and Noller, 1979). Proteins L18 and L25 have been mapped by immunoelectron microscopy at the central protuberance (Stöffler-

Meilicke et al., 1983b; Noah, 1982). The 3'-end of the 5S RNA has also been localized at the central protuberance (Shatsky et al., 1980; Stöffler-Meilicke et al., 1981). Thus, the localization of protein L5 completes the topography of a structural domain of the 50S subunit (Fig.51a). It has been proposed that a fourth protein, namely L31, also binds to the 5S RNA (Fanning and Traut, 1981). Protein L31 has not yet been localized but its proximity with L5 is supported by cross-linking results (Cover et al., 1981). L5 has also been crosslinked to proteins L11 and L7/L12 (Table 14). Since these proteins have been localized at or at the base of the stalk (Fig.51a), they seem to be too far away from L5 to be crosslinked to it, the maximum length of the cross-linking agent being 24 A°. However, one has to consider that those parts of the two proteins determined by immunoelectron microscopy need not be the same as those crosslinked with bifunctional reagents and it cannot be excluded that other regions of the proteins may be closer to each other. The cross-links of L5 with protein L23 and L17 are not compatible with our model (Fig.51a).

Our mapping at the interface between the two subunits agrees well with the cross-linking of L5 with proteins S9, S13 and S19 (Table 14). Proteins S13 and S19 have been located at the head of the 30S subunit (Stöffler-Meilicke and Stöffler, 1984). S9 has also been placed in the same region by neutron scattering (Ramakrishan et al., 1981). Thus, these three proteins are neighbours of L5 in the model of the 70S ribosome.

6.5.6. Protein L27

Protein L27 has been localized in the middle of the interface side of the 50S subunit, in a region extending from the lower part of the central protuberance to the base of the particle (Fig.51a).

Additional information about the localization of L27 was obtained by reconstitution of 50S subunits of a mutant of Escherichia coli which lacks protein L27, with BL27 from Bacillus subtilis and subsequent localization of BL27 by the corresponding antibody. It has been shown that a protein can be assembled in mutant ribosomes from another bacterial specie by a one-step reconstitution procedure. This approach should allow the localization of some of the proteins which have not yet been mapped because of the lack of reactive antibodies. For example, a mutant of E. coli which lacks protein S20 has been isolated (Dabbs, 1979) and in our laboratory there are available reactive IgGs specific for protein S20 of Bacillus stearothermophilus. Thus, it should be possible to assemble protein BS20 in the mutant ribosomes and to map it by the corresponding antibody. The same strategy could be employed for proteins S9, L28 and L30, since mutants which lack these proteins have been isolated (Dabbs et al., 1983). Another possibility is to assemble in the mutant ribosomes a haptenated protein and to localize it by hapten-specific antibodies. Up to now, for this kind of study a complete reconstitution procedure has been used (see for example, Stöffler-Meilicke et al., 1983a). An important advantage of this approach is that it provides an optimal control for the accuracy of protein mapping, since crossreactions with other proteins of E. coli can be excluded (see also Fig.48). In our experiments on L27, we observed that the reactivity of the reconstituted subunits with anti-BL27 was slightly lower than the reactivity of 50S subunits from Bacillus subtilis. Electron micrographs of the latter immunocomplexes showed that the anti-BL27 preparation contained contaminating antibodies reacting with other ribosomal proteins of the 50S subunit of B. subtilis. The occurrence of such contaminants was not significant for our study (5.8.6.). Thus, our results indicated that the reconstitution occurred with high effectiveness in spite of the evolutionary distance between the two organisms.

The comparison of the electron micrographs obtained by reacting 50S subunits of E. coli with anti-L27 and of reconstituted subunits with anti-BL27 revealed some differences in the location of the two epitopes. When the subunits were seen in the crown projection, pairs in which the two particles were very close were seen only with anti-L27 (Fig.43b). Immunocomplexes in which antibody binding was at the central protuberance have been observed with both antibodies (compare Fig.43d with Fig.49d). Complexes in which the central protuberances of the two subunits were facing each other are characteristic for the only anti-BL27 (Fig.49c). The more relevant discrepancy concerned the antibody binding site to the kidney projectional form, anti-BL27 attaching to the convex side of the particle (Fig.49e); however, the antibody binding site proposed is still consistent with a location of the epitope at the base of the central protuberance (Fig.45). The slight differences observed in the location of L27 and BL27 can be explained by differences in the position of the reactive epitopes. The same was found in a comparative study on the localization of protein S4 on E. coli and B. stearothermophilus 30S subunits (Stöffler-Meilicke et al., 1984).

Moreover, even when the same protein is localized by different antibodies, differences in the antibody binding sites can be observed, depending on the specificity of different IgGs for different antigenic determinants. For example, two anti-L1 preparations used for immunoelectron microscopy were found to be significantly different. One antibody bound exclusively to the top of the L1 protuberance and two antibodies bound simultaneously were rarely observed. The other antibody bound in the whole region of the L1 protuberance and yielded a high percentage of subunits which were simultaneously joined by two IgG molecules.

In the case of protein L27 it was possible to compare our

localization with the results of another immunoelectron microscopic study (Lake and Strycharz, 1981). These authors localized L27 at the top of the central protuberance. This finding does not completely agree with our own results, which indicate that L27 is situated rather towards the middle part of the subunit. Our placement is also supported by the observation that anti-L27 antibodies inhibit the binding of IgG specific for protein L19 which has been located on the base of the 50S subunit (5.8.5.). We concluded that these two proteins are close to each other. Evidence that L19 and L27 are neighbours in the 50S particle is provided by the finding that the antibiotic chloramphenicol can be crosslinked to both proteins (Wilchek, personal communication). A localization of protein L27 at the ribosomal interface is also consistent with the obervation that anti-L27 IgGs do not react with 70S ribosomes (5.8.4.) and that they inhibit the association of the subunits (Highland et al., 1974).

L27 is a quite small protein with a molecular weight of 8.993 daltons (Wittmann, 1982). Gel filtration experiments showed that in solution it has an extended shape (Dijk and Wills, personal communication). Thus, although not very likely, it cannot be excluded that L27 might cover a relatively broad area of the subunit and that different epitopes of L27 have been mapped by Lake and by ourselves.

L27 has been crosslinked with proteins L16, L17, L29 and S9 (Table 14). Our localization of L27 at the interface is consistent with the L27-S9 crossink. L16 has not yet been mapped but, since it has been shown to be important for the peptidyltransferase activity (Nierhaus and Montejo, 1973) it is likely that the two proteins are neighbours in the 50S subunit. On the contrary, the crosslinks with L17 and L29 are not compatible with our model since these two proteins have been localized on the back of the subunit (Fig.51a). Our localization fits well the existence of RNA-protein complexes with

protein L15 (Table 14).

Protein L27 has been described as important for peptidyltransferase activity (Nierhaus, 1982; Liljas, 1982), and this would be consistent with its localization. However, the viability of mutants lacking protein L27 rules out that this protein is essential for this function.

6.6. Location of the 50S subunit proteins in the 70S ribosome

Finally, we investigated the location of ribosomal proteins on the complete 70S ribosome, in order to have more information about the structure of the functioning ribosome. When antibodies against 50S subunit proteins were reacted with 70S ribosomes, anti-L5, anti-L19 and anti-L27 were not reactive, whereas all other antibodies formed 70S-IgG-70S complexes (Fig.52). From these data it could be concluded that proteins L5, L19 and L27 have antigenic determinants on the surface of the 50S subunit, but not on the surface of the 70S ribosome. These results agree with the localization of these proteins on the interface side of the 50S subunit.

This investigation was also helpful in determining the actual position of those proteins which are situated close to the interface, like L6 and L9. The reactivity of anti-L6 and anti-L9 antibodies with 70S ribosomes, showed that the antigenic determinants of these two proteins are not covered by the 30S particle upon association of the two subunits (Fig.52). Some electron micrographs of anti-L5 and anti-L18 immunocomplexes look very similar, both antibodies binding to the central protuberance. We have localized L5 below protein L18 (Fig.51a). The observation that the reactive epitope of L5 is not accessible to antibody binding in the 70S monosome, whereas L18 is still accessible, confirmed the relative locations of the two

still accessible, confirmed the relative locations of the two proteins (Fig.51a).

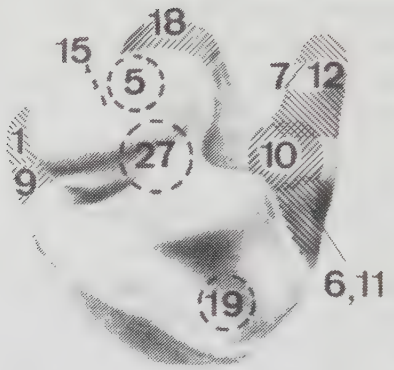


Fig.52. Three-dimensional model of the 70S ribosome showing the locations of the 50S subunit proteins. The dotted circles indicate proteins which are not accessible for antibody binding in the 70S monosome. Proteins L4, L17, L27 and L29 have been localized on the back of the subunit.

7. REFERENCES

Atsmon A., Spitnik-Elson P., and Elson D. (1969). J. Mol. Biol. 45:125-135

Boublik M., Hellmann W., and Kleinschmidt A.K. (1977). Cytobiologie 14:293-300

Boublik M., Spiess E., Roth H.E., Hellmann W., and Jenkins F. (1978). Cytobiologie 18:309-319

Boublik M., Robakis N., Hellmann W., and Wall J.S. (1982). Eur. J. of Cell. Biol. 27:177-184

Brimacombe R., Maly P., and Zwieb C. (1983). Progr. in Nucl. Acid Res. and Mol. Biol. 28:1-48

Chang F.N. and Flaks J.G. (1971). J. Mol. Biol. 61:387-400

Chen-Schmeisser U. and Garrett R.A. (1976). Eur. J. Biochem. 69:401-410

Cover J.A., Lambert J.M., Normann C.M., and Traut R.R. (1981). Biochemistry 20:2843-2852

Craven G.R. and Gupta V. (1970). Proc. Natl. Acad. Sci. USA 67:1329-1336

Crichton R.R. and Wittmann H.G. (1971). Mol. Gen. Genet. 114:95-105

Dabbs E.R. (1979). J. of Bacteriol. 140:734-737

Dabbs E.R., Ehrlich R., Hasenbank R., Schröter B.H., Stöffler-Meilicke M., and Stöffler G. (1981). J. Mol. Biol. 149:553-578

Dabbs E.R., Hasenbank R., Kastner B., Rak K.-H., Wartusch B., and Stöffler G. (1983). Mol. Gen. Genet. 192:301-308

Dijk J. and Littlechild J. (1979). Meth. Enzymol. 9:481-502

Eisen H.N. (1973), In: Microbiology. Eds: Davis B.D., Dulbecco R., Eisen H.N., Ginsberg H.S., Wood W.B.. Harper International Edition Medical Dept. (Harper and Row Publishers, Hagerstown, Maryland, New York, Evanston, San Francisco, London, p.349

Erdmann V.A. (1976). In: Cohn W.E. (ed.) Progr. in Nucleic Acid Res. and Mol. Biol., Academic Press, New York 18:45-90

Fanning T.G. and Traut R.R. (1981). Nucl. Acid Res. 9:993-1004

Finch J.T. and Klug A. (1965). J. Mol. Biol. 13:1-12

Garrett R.A. and Noller H.F. (1979). J. Mol. Biol. 132:637-648

Giri L., Hill W.E., and Wittmann H.G. (1984). Adv. in Prot. Chem. 36:1-55

Girshovich A.S., Kurtskalia T.V., Ovchinnikov Y.A., and Vasiliev V.D. (1981). FEBS Letters 130:54-59

Hardy S.J.S., Kurland C.G., Voynow P., and Mora G. (1969). Biochemistry 8:2897-2905

Highland J.H., Ochsner E., Gordon J., Bodley J.W., Hasenbank R., and Stöffler G. (1974). Proc. Natl. Acad. Sci. USA 71:627-630

Hindennach I., Stöffler G., and Wittmann H.G. (1971). Eur. J. Biochem. 23:7-11

Homann H.E. and Nierhaus K.H. (1971). Eur. J. Biochem. 20:249-257

Huxley H.E. and Zubay G. (1960). J. Mol. Biol. 2:10-18

Kahan L. and Kaltschmidt E. (1972). Biochemistry 11:2691-2698

Kahan L., Winkelmann D.A., and Lake J.A. (1981). J. Mol. Biol. 145: 193-214

Kaltschmidt E. and Wittmann H.G. (1970). Proc. Natl. Acad. Sci. USA 67:1276-1282

Kastner B., Stöffler-Meilicke M., and Stöffler G. (1981). Proc. Natl. Acad. Sci. USA 78:6652-6656

Lake J.A. (1976). J. Mol. Biol. 105:131-159

Lake J.A. (1978). In: Advanced Techniques in Biological Electron Microscopy II. Springer Verlag, Berlin, Heildelberg, New York, pp: 173-211

Lake J.A. (1980). In: Chambliss C., Craven G.R., Davies G., Kahan L., Nomura M. (eds). Ribosomes. Structure, function and genetics. University Park Press, Baltimore, pp: 207-236

Lake J.A., Pendergast M., Kahan L., and Nomura M. (1974). Proc. Natl. Acad. Sci. USA 71:4688-4692

Lake J.A. and Strycharz W.A. (1981). J. Mol. Biol. 153:979-992

Leboy P.S., Cox E.C., and Flask J.G. (1964). Proc. Natl. Acad. Sci. USA 52:1367-1374

Lelong J.C., Maschler R., Crepin M., Jeantet C., Tischendorf G.W., and Gross F. (1979). Biochimie 61:881-889

Liljas A. (1982). Prog. Biophys. Molec. Biol. 40:161-228

Litman D.J. and Cantor C.R. (1974). Biochemistry 13:512-518

Lotti M., Dabbs E.R., Hasenbank R., Stöffler-Meilicke M., and Stöffler G. (1983). Mol. Gen. Genet. 192:295-300

Lührmann R. (1976). Dissertation, Munster

Lührmann R., Bald R., Stöffler-Meilicke M., and Stöffler G. (1981). Proc. Natl. Acad. Sci. USA 78:7276-7280

Mc-Kuskie-Olson H., Grant P.G., Cooperman B.S., and Glitz D. (1982). J. Biol. Chem. 257:2649-2656

Meisenberger O., Pilz I., Stöffler-Meilicke M., and Stöffler G. (1984). Biochim. Biophys. Acta 781:225-233

Mizushima S. and Nomura M. (1970). Nature 226:1214-1218

Moore P.B. (1971). J. Mol. Biol. 60:169-184

Moore P.B. (1980). In: Chambliss C., Craven G.R., Davies G., Kahan L., Nomura M. (eds). Ribosomes. Structure, function and genetics. University Park Press, Baltimore, pp:111-133

Morrison C.A., Tischendorf G.W., Stöffler G., and Garrett R.A. (1977). Mol. Gen. Genet. 151:245-252

Myazawa Y., Hosoi J., and Mochizuki A. (1979). J. Electron Microsc. 28:308-311

Newton I. and Brimacombe R. (1974). Eur. J. Biochem. 48:513-518

Nierhaus K.H. (1980). In: Chambliss C., Craven G.R., Davies G., Kahan L., Nomura M. (eds). Ribosomes. Structure, function and genetics. University Park Press, Baltimore, pp:267-294

Nierhaus K.H. (1982). In: Henle W., Hofschneider P.H., Koprowsky H., Melchers F., Rott R., Schweiger H.G., Vogt P.K. (eds). Current Topics in Microbiology and Immunology 97:91-155

Nierhaus K.H. and Montejo V. (1973). Proc. Natl. Acad. Sci. USA 70:1931-1935

Nierhaus K.H., Lietzke R., May P.R., Nowotny V., Schulze H., Simpson K., Wurmbach P., and Stuhrmann H.B. (1983). Proc. Natl. Acad. Sci. USA 80:2889-2893

Noah M. (1982). Dissertation. Technische Universität. Berlin

Noll M., Hapke B., Schreier M.H., and Noll H. (1973). J. Mol. Biol. 75:281-294

Noller H.F. (1984). Ann. Rev. Biochem. 53:119-162

Österberg R., Sjöberg B., Garrett R., and Littlechild J. (1974). FEBS Letters 73:25-28

Pettersson I. and Liljas A. (1979). FEBS Letters 98:139-144

Pieler T. and Erdmann V.A. (1982). Proc. Natl. Acad. Sci. USA 79:4599-4603

Ramakrishnan V.R., Yabuki S., Sillers I.-Y., Schindler D.G., Engelman D.M., and Moore P.B. (1981). J. Mol. Biol. 153:739-760

Ramakrishnan V.R., Capel M., Kjelgaard M., Engelman D.M., Moore P.B. (1984). J. Mol. Biol. 174:265-284

Randall-Hazelbauer L. and Kurland C. G. (1972). Mol. Gen. Genet. 115:234-242

Röhl R. and Nierhaus K.H. (1982). Proc. Natl. Acad. Sci. USA 79:729-733

Rohde M.F., O'Brien S., Cooper S., and Aune K.C. (1975). Biochemistry 14:1079-1087

Roth H.E. and Nierhaus K.H. (1975). J. Mol. Biol. 94:111-121

Schwedler-Breitenreuter G., Lotti M., Stöffler-Meilicke M., and Stöffler G. (1985). In press

Sela M. (1969). Science 166:1365-1374

Serdyuk I.N., Grenader A.K., and Zaccai G. (1979). J. Mol. Biol. 135:691-707

Serdyuk I.N., Agalarov S.C., Sedelnikova S.E., and Spirin A.S. (1983) J. Mol. Biol. 169:409-425

Shatsky I.N., Evastafieva A.G., Bystrova T.F., Bogdanov A.A., and Vasiliev V.D. (1980). FEBS Letters 121:97-100

Sherton C.C. and Wool I.G. (1972). J. Biol. Chem. 247:4460-4467

Spinik-Elson P. and Breiman A. (1971). Biochim. Biophhys. Acta 254:457-467

Stöffler-Meilicke M., Stöffler G., Odom O.W., Zinn A., Kramer G., and Hardesty B. (1981). Proc. Natl. Acad. Sci. USA 78:5538-5542

Stöffler-Meilicke M., Epe B., Steinhäuser K.G., Woolley P., and Stöffler G. (1983a). FEBS Letters 163:94-98

Stöffler-Meilicke M., Noah M., and Stöffler G. (1983b). Proc. Natl. Acad. Sci. USA 80:6780-6784

Stöffler-Meilicke M. and Stöffler G. (1984). In: Csnady A., Röhlich P. and Szabo D. (eds), Proceedings of the Eighth European Congress on Electron Microscopy, Budapest, pp 1525-1536

Stöffler-Meilicke M., Epe B., Woolley P., Lotti M., Littlechild J., and Stöffler G. (1984). Mol. Gen. Genet. 197:8-18

Stöffler G. (1967). Mol. Gen. Genet. 100:374-377

Stöffler G. (1974). In: Nomura M., Tissieres A., Lengyel P. (eds). Ribosomes. Long Island NY. Cold Spring Harbor L., pp: 615-667

Stöffler G. and Wittmann H.G. (1971a). Proc. Natl. Acad. Sci. USA 68:2283-2287

Stöffler G. and Wittmann H.G. (1971b). J. Mol. Biol. 62: 407-409

Stöffler G., Hasenbank R., Lütgehaus M., Maschler R., Morrison C.A., Zeichhardt H., and Garrett R.A. (1973). Mol. Gen. Genet. 127:89-100

Stöffler G., Tate W.P., and Kaskey C.T. (1982). J. Biol. Chem. 257: 4203-4206

Stöffler G. and Stöffler-Meilicke M. (1984). Ann. Rev. Biophys. Bioeng. 13:303-330

Stöffler G., Noah M., Stöffler-Meilicke M., and Dabbs E.R. (1984). J. Biol. Chem. 259:4521-4526

Terhost C., Möller W., Laursen R., and Wittmann-Liebold B. (1972). FEBS Letters 28:325-328

Tischendorf G.W., Zeichhardt H., and Stöffler G. (1974a). Mol. Gen. Genet. 134:187-208

Tischendorf G.W., Zeichhardt H., and Stöffler G. (1974b). Mol. Gen. Genet. 134:209-223

Tischendorf G.W. and Stöffler G. (1975). Mol. Gen. Genet. 142:193-208

Tischendorf G.W., Zeichhardt H, and Stöffler G. (1975). Proc. Natl. Acad. Sci. USA 72:4820-4824

Towbin H., Staehelin T., and Gordon G. (1979). Proc. Natl. Acad. Sci. USA 76:4350-4354

Traut R.R., Lambert J.M., Boileau G, and Kenny J.W. (1980). In: Chambliss C., Craven G.R., Davies G., Kahan L., Nomura M. (eds) Ribosomes. structure, function and genetics. University Park Press, Baltimore, pp: 89-110

Traut R.R., Lambert J.M., and Kenny J.W. (1983). J. Biol. Chem. 258: 4592-4598

Vasiliev V.D. and Koteliansky V.E. (1977). FEBS Letters 76:125-128

Vasiliev V.D., Koteliansky V.E.,and Rezapkin G.V. (1977a). FEBS Letters 79:170-174

Vasiliev V.D., Koteliansky V.E., Shatsky I.N., and Rezapkin G.V. (1977b). FEBS Letters 84:43-47

Vasiliev V.D., Selivanova O.M., and Kotenliansky V.E. (1978). FEBS Letters 95:273-276

Vasiliev V.D. and Zalite O.M. (1980). FEBS Letters 121:101-104

Vasiliev V.D., Selivanova O.M., Baranov V.I., and Spirin A.S. (1983). FEBS Letters 155:167-172

Wabl M.R. (1973). Dissertation. Freie Universität. Berlin

Whiting R.F. and Ottensmeyer F.P. (1972). J. Mol. Biol. 67:173-181

Winkelmann D. and Kahan L. (1979). J. Supr. St. C.10:443-455

Winkelmann D.A., Kahan L., and Lake J.A. (1982). Proc. Natl. Acad. Sci. USA 79:5184-5188

Winkelmann D.A. and Kahan L. (1983). J. Mol. Biol. 165:357-374

Wittmann-Liebold B. and Dzionara M. (1976). FEBS Letters 65:281-283

Wittmann H.G. (1982). Ann, Rev, Biochem. 51:155-183

Wittmann H.G. (1983). Ann. Rev. Biochem. 52:35-65

Wittmann H.G., Littlechild J.A., and Wittmann-Liebold B. (1980). In: Chambliss C., Craven G.R., Davies G., Kahan L., Nomura M. (eds). Ribosomes. Structure, function and genetics. University Park Press, Baltimore, pp:51-88

Wong K.P. and Paradies H.H. (1974). Biochem. Biophys. Res. Commun. 61:178-184

Wystup G., Teraoka H., Schulze H., Hampl H., and Nierhaus K.H. (1979) Eur. J. Biochem. 100:101-113

Zeichhardt H. (1976). Dissertation, Freie Universität, Berlin

Zubke W., Staedler H., Ehrlich R., Stöffler G., Wittmann H.G., and Apirion D. (1977). Mol. Gen. Genet. 158:129-139

DANKSAGUNG

Diese Arbeit wurde am Max-Planck-Institut für Molekulare Genetik (Berlin-Dahlem), Abteilung Wittmann, in der Arbeitsgruppe von Herrn Prof. Dr. G. Stöffler angefertigt.

Frau Dr. Marina Stöffler-Meilicke und Herrn Prof. Georg Stöffler danke ich für ihre Unterstützung und ihr Interesse an meiner Arbeit.

Für ihre Hilfsbereitschaft und für die schöne Atmosphäre möchte ich mich bei allen Mitgliedern unserer Arbeitsgruppe: R. Albrecht, C. Böhme, R. Hasenbank, B. Kastner, G. Schwedler-Breitenreuter, L. Schuster und K. Rak, und bei allen meinen Freunden im Institut bedanken.

Herrn G. Haraux danke ich für die schnelle und sorgfältige Korrektur des Manuskriptes.

LEBENSLAUF

Name:	Lotti, Marina
Geburtstag und Ort:	21 Juli 1957, Mailand, Italien
1963-1970:	Grundschule in Mailand
1970-1975:	Gymnasium A. Manzoni in Mailand
Juli 1975:	Abitur (Maturita' classica)
Nov 1976:	Erstimmatrikulation an der Universität Mailand am Fachbereich Biologie
1979-1980:	Durchführung der Diplomarbeit (Tesi di Laurea) mit dem Thema: "Elektronenmikroskopische, autoradiographische und cytochemische Untersuchung des OSDV Virus" am Institut für Botanik der Universität Mailand unter der Leitung von Prof. M.A. Favali and Prof. F.M. Gerola
Nov. 1980:	Diplomprüfung (Esame di Laurea) mit Auszeichnung
1981:	Wissenschaftliche Mitarbeit am Institut für Botanik der Universität Mailand
Seit März 1982:	Anfertigung der Dissertation am Max-Planck Institut für Molekulare Genetik, Berlin, Abteilung Wittmann, bei Priv. Doz. Dr. M. Stöffler-Meilicke